

# JOURNAL OF AGRICULTURAL RESEARCH

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## CONTENTS

	Page
Anatomy and Metamorphosis of the Apple Maggot <i>Rhagoletis pomonella</i> Walsh. R. E. SNODGRASS (8 text figures; 6 plates)-----	1
Chemical and Biological Studies with Cyanamid and Some of its Transformation Products. K. D. JACOB, F. E. ALLISON and J. M. BRAHAM (12 test figures)-----	37
<i>Grossularia echinella</i> , a Spiny-Fruited Gooseberry from Florida. FREDERICK V. COVILLE (1 plate)-----	71
Fowl Typhoid, its Dissemination and Control. B. F. KAUPP and R. S. DEARSTYNE (2 text figures)-----	75
Effects of the Modified Hot-Water Treatment on Germination, Growth and Yield of Wheat. V. F. TAPKE (5 plates)-----	79
Types of Vegetation in the Semiarid Portion of the United States, and their Economic Significance. A. E. ALDOUS and H. L. SHANTZ (1 map; 16 plates)-----	99
The Development of American Foulbrood in Relation to the Metabolism of its Causative Organism. A. P. STURTEVANT (17 text figures)-----	129
Brittle Straw and Other Abnormalities in Rye. F. R. DAVIDSON, H. E. BREWBAKER and N. A. THOMPSON (2 text figures)-----	169
Relation between Toxicity of Cottonseed and its Gossypol Content. ERICH W. SCHWARTZE and CARL L. ALSBERG (13 text figures)-----	173 ✓
Pharmacology of Gossypol. ERICH W. SCHWARTZE and CARL L. ALSBERG (2 plates)-----	191 ✓
The Relation of Antecedent Egg Production to the Sex Ratio of the Domestic Fowl. M. A. JULL-----	199
A Gradient of Permeability to Iodin in Wheat Seed Coats. HARRY BRAUN-----	225
Further Studies on the Relative Susceptibility to Citrus Canker of Different Species and Hybrids of the Genus Citrus, Including the Wild Relatives. GEORGE L. PELTIER and WILLIAM J. FREDERICH-----	227
Relation of Environmental Factors to Citrus Scab Caused by <i>Cladosporium citri</i> Masse. GEORGE L. PELTIER and WILLIAM J. FREDERICH (2 text figures)-----	241
A Study of Ensiling a Mixture of Sudan Grass with a Legume. P. A. WRIGHT and R. H. SHAW-----	255 ✓
Bacterial Leafspot of Delphinium. MARY K. BRYAN (4 plates)-----	261
Further Observations on the Molts of the Ox Bots <i>Hypoderma bovis</i> De Geer and <i>H. lineatum</i> Villers. E. W. LAAKE-----	271
<i>Flavobacterium suaveolens</i> , a New Species of Aromatic Bacillus Isolated from Dairy Wastes. LULU SOPPELAND-----	275 ✓
Some Relations between the Growth and Composition of Young Orange Trees and the Concentration of the Nutrient Solution Employed. H. S. REED and A. R. C. HAAS (1 plate)-----	277
The Germination of Cottonseed. EBEN H. TOOLE and PEARLE L. DRUMMOND (2 plates)-----	285
The Salt Content of Cotton Fiber. THOMAS H. KEARNEY and C. S. SCOFIELD-----	293
Studies on Curly-Top Disease of the Sugar Beet. EUBANKS CARSNER and C. F. STAHL (1 text figure; 5 plates)-----	297
Pecan Scab with Special Reference to Sources of the Early Spring Infections. J. B. DEMAREE (2 plates)-----	321
Anthelmintic Efficiency of Carbon Tetrachlorid in the Treatment of Foxes. KARL B. HANSON and H. L. VAN VOLKENBERG-----	331
The Chemical Composition of Edible Viscera from Meat-Producing Animals. WILMER C. POWICK and RALPH HOAGLAND-----	339
Fumigation of Bean Weevils, <i>Bruchus obtectus</i> Say and <i>B. quadrimaculatus</i> Fab. A. O. LARSON-----	347

	Page
Leather Rot of Strawberries. DEAN H. ROSE (6 text figures; 2 plates) ..	357
The Cambium Curculio, <i>Conotrachelus anaglypticus</i> Say. FRED E. BROOKS and R. T. COTTON (1 text figure; 3 plates) ..	377
Relation of the Molecular Proportions in the Nutrient Solution to the Growth of Wheat. M. C. SEWELL (4 text figures) ..	387
Studies on Nonarsenical Stomach-Poison Insecticides. WILLIAM MOORE and F. L. CAMPBELL ..	395
Studies upon the Relative Merits of Sweet Corn Varieties for Canning Purposes and the Relation of Maturity of Corn to the Quality of the Canned Product. C. W. CULPEPPER and C. A. MAGOON (11 text figures) ..	403
The Photoperiodism of <i>Tephrosia candida</i> . T. B. MCCLELLAND (7 text figures; 4 plates) ..	445
Antineuritic Vitamin in Poultry Flesh and Eggs. RALPH HOAGLAND and ALFRED R. LEE ..	461
Dissemination of the Stem and Bulb Infesting Nematode, <i>Tylenchus dipsaci</i> , in the Seeds of Certain Composites. G. H. GODFREY (3 plates) ..	473
Some Modifications of the Picric Acid Method for Sugars. J. J. WILLA- MAN and F. R. DAVISON (2 text figures) ..	479
Effect of Moisture on a Seed-Borne Bean Disease. LEWIS T. LEONARD (4 text figures) ..	489
Factors Influencing the Binding Power of Soil Colloids. HOWARD E. MIDDLETON (6 text figures) ..	499
The Genetic Relation between <i>Triticum dicoccum dicoccoides</i> and a Similar Morphological Type Produced Synthetically. H. H. LOVE and W. T. CRAIG (8 plates) ..	515
Bud Selection as Related to Quality of Crop in the Washington Navel Orange. A. D. SHAMEL, C. S. POMEROY, and R. E. CARYL (4 plates) ..	521
✓ The Determination of Nitrate and Ammonia in Nitrogenous Materials. O. M. SHEDD ..	527
<i>Puccinia Graminis Poae</i> Erikss. and Henn. in the United States. E. C. STAKMAN and M. N. LEVINE (2 text figures; 1 plate) ..	541
Relation of Soil Temperature and Soil Moisture to Infection by <i>Plas- modiophora brassicae</i> . JOHN MONTEITH, Jr. (5 plates) ..	549
Length of Cotton Fiber from Bolls at Different Heights on the Plant. THOMAS H. KEARNEY and GEORGE J. HARRISON (1 text figure) ..	563
"Hairy Neck" Wheat Segregates from Wheat-Rye Hybrids. CLYDE E. LEIGHTY and J. W. TAYLOR (5 plates) ..	567
Ascaris Sensitization. B. H. RANSOM, W. T. HARRISON, and J. F. COUCH (1 plate) ..	577
<i>Physalospora malorum</i> on Currant. NEIL E. STEVENS (2 plates) ..	583
Botryosphaeria and Physalospora on Currant and Apple. C. L. SHEAR, NEIL E. STEVENS, and MARGUERITE S. WILCOX (3 text figures; 2 plates) ..	589
Isolation of an Inhibitory Substance from Plants. W. L. MALLMAN and CARL HEMSTREET ..	599
Two Hitherto Unreported Diseases of Stone Fruits. C. C. LINDEGREN and DEAN H. ROSE ..	603
Studies Relating to the Immunology of Bovine Infectious Abortion. J. M. BUCK and G. T. CREECH ..	607
The Rhizoctonia Brown Rot and Other Fruit Rots of Strawberries. B. O. DODGE and NEIL E. STEVENS (3 plates) ..	643
Effective Use of Hydrocyanic-Acid Gas in the Protection of Chick-Peas ( <i>Cicer arietinum</i> ) Warehoused in 240-Pound Sacks. E. A. BACK and R. T. COTTON (1 text figure; 1 plate) ..	649
A New Nematode, <i>Cylindropharynx ornata</i> , from the Zebra, with Keys to Related Nematode Parasites of the Equidae. ELOISE B. CRAM (8 text figures) ..	661
Chromosomes in Maize and Maize Relatives. ALBERT E. LONGLEY (3 plates) ..	673
A Fusarium Bulb Rot of Onion and the Relation of Environment to Its Development. J. C. WALKER and E. C. TIMS (3 text figures; 3 plates) ..	683
The Chlorid Content of the Leaf Tissue Fluids of Egyptian and Upland Cotton. J. ARTHUR HARRIS, JOHN V. LAWRENCE, and ZONJA WALLEN LAWRENCE ..	695



	Page
The Resistance of Oat Varieties to Stem Rust. WILLIAM W. MACKIE and RUTH F. ALLEN (1 text figure; 2 plates)-----	705
Indicator Significance of the Natural Vegetation of the Southwestern Desert Region. H. L. SHANTZ and R. L. PIEMEISEL (21 text figures; 14 plates)-----	721
Acromania, or "Crazy-Top," a Growth Disorder of Cotton. O. F. COOK (15 plates)-----	803
The Polyembryonic Development of <i>Platygyaster vernalis</i> . R. W. LEIBY and C. C. HILL (8 plates)-----	829
The Inheritance of Pubescent Nodes in a Cross between Two Varieties of Wheat. H. H. LOVE and W. T. CRAIG-----	841
Density of Cell Sap in Relation to Environmental Conditions in the Wasatch Mountains of Utah. C. F. KORSTAIN (5 text figures)-----	845
An Ascigerous Stage and Synonymy for <i>Fusarium moniliforme</i> . GRACE O. WINELAND (2 plates; 6 text figures)-----	909
Expulsion of Aecidiospores by the Mayapple Rust, <i>Puccinia podophylli</i> Schw. B. O. DODGE (1 plate; 1 text figure)-----	923
The Heat of Wetting of Soil Colloids. M. S. ANDERSON-----	927
Comparison of Pima Cotton with Upland Varieties in Arizona. C. J. KING, H. F. LOOMIS, and D. L. VARMETTE (3 plates; 4 text figures)-----	937
The Fungus Causing the Common Brown Rot of Fruits in America. JOHN W. ROBERTS and JOHN C. DUNEGAN (2 plates)-----	955
Preliminary Results with the Borax Treatment of Citrus Fruits for the Prevention of Blue Mold Rot. HARRY R. FULTON and JOHN J. BOWMAN (5 text figures)-----	961
Snails as Predisposing Agents of Sugar Cane "Root Disease" in Louisiana. R. D. RANDS-----	969
Greenhouse Experiments with Atmospheric Nitrogen Fertilizers and Related Compounds. F. E. ALLISON, E. B. VLIET, J. J. SKINNER, and F. R. REID (1 plate)-----	971
Individual and Age Variation in <i>Microtus montanus yosemite</i> . A. BRAZIER HOWELL (1 plate; 25 text figures)-----	977
A Genetic and Cytological Study of Certain Hybrids of Wheat Species. KARL SAX and E. F. GAINES (2 plates)-----	1017
Determination of Temperatures Fatal to the Powder-Post Beetle, <i>Lyctus planicollis</i> Leconte, by Steaming Infested Ash and Oak Lumber in a Kiln. T. E. SNYDER and R. A. ST. GEORGE (1 plate)-----	1033
Bacterial Blight of Rye. C. S. REDDY, JAMES GODKIN, and A. G. JOHNSON (1 plate)-----	1039
Non-Inheritance of Terminal Bud Abortion in Pima Cotton. THOMAS H. KEARNEY (1 plate)-----	1041
Relative Resistance of the Rice Weevil, <i>Sitophilus oryza</i> L., and the Granary Weevil, <i>S. granarius</i> L., to High and Low Temperatures. E. A. BACK and R. T. COTTON-----	1043
Uninucleated Aecidiospores in <i>Caeoma nitens</i> , and Associated Phenomena. B. O. DODGE (5 plates)-----	1045
On Some Plant Parasitic Nemas and Related Forms. G. STEINER. (4 plates)-----	1059
The Influence of Environment on Sex in Hemp, <i>Cannabis sativa</i> L. HUGH C. MCPHEE (1 plate; 3 text figures)-----	1067
Spacing Experiments with Acala Cotton in Southern California. H. G. MCKEEVER (3 text figures)-----	1081
Correlation Among Quantitative Characters in Maize. J. H. KEMPTON (1 plate; 5 text figures)-----	1095
Effect of Fumigation upon Heating of Grain Caused by Insects. E. A. BACK and R. T. COTTON (2 text figures)-----	1103
The Nitrification of Phosphorus Nitride. F. E. ALLISON-----	1117
Aecial Stages of the Leaf Rusts of Rye, <i>Puccinia dispersa</i> Erikss. and Henn., and of Barley, <i>P. anomala</i> Rostr., in the United States. E. B. MAINS and H. S. JACKSON (1 plate)-----	1119
Fall Sowing and Delayed Germination of Western White Pine Seed. W. G. WAHLENBERG (4 text figures)-----	1127
The European Hare ( <i>Lepus europaeus</i> Pallas) in North America. JAMES SILVER (1 text figure)-----	1133
Growth on Cut-Over and Virgin Western Yellow Pine Lands in Central Idaho. C. F. KORSTIAN (3 plates)-----	1139

	Page
Some Factors Affecting Reproduction after Logging in Northern Idaho. J. A. LARSEN (5 text figures)-----	1149
The Effect of Cyanamid and Related Compounds on the Number of Microorganisms in Soil. F. E. ALLISON-----	1159
Morphology of the Honeybee Larva. JAMES A NELSON (8 plates; 5 text figures)-----	1167
Effect of Winter Rations on Subsequent Pasture Gains of Steers. E. W. SHEETS (9 plates; 12 text figures)-----	1215
A Study of Douglas Fir Reproduction under Various Cutting Methods. JACOB ROESER, Jr. (1 plate; 3 text figures)-----	1233
The Isolation and Identification of Quercetin from Apple Peels. CHARLES E. SANDO-----	1243
Gamete Production in Certain Crosses with "Rogues" in Peas. WILBER BROTHERTON, Jr.-----	1247
Survey of Blister Rust Infection on Pines at Kittery Point, Maine, and the Effect of Ribes Eradication in Controlling the Disease. G. B. POSEY and E. R. FORD (5 text figures)-----	1253

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## ERRATA AND AUTHOR'S EMENDATIONS

- Page 3, line 3, the second word "hooks" should be "teeth."  
 Page 23, line 26, "Pl. 5, D, N<sub>2</sub>" should read "Pl. 5, E, N<sub>1</sub>."  
 In "Types of Vegetation in the Semiarid Portion of the United States": Wherever "wire needleglass" is used "wire grass" should be substituted.  
 Page 114-A, line 3, in Type 89, eliminate "probably in."  
 Page 124, insert type 22 (wire grass) in Region I.  
 Page 125, eliminate "Type 22, wire grass," and "31 (blackgrama and wire grass)" from Region 8.  
 Page 126, insert "type 71 (Encelia and California sagebrush)" in Region 8.  
 Page 129, in the title of author "Agricultural" should read "Apicultural".  
 Page 257, line 10, "Sudan grass" should read "soy beans."  
 Page 272, Table I, column 12, "minimum" should read "maximum."  
 Page 295, line 17, "absorber" should read "absorbed."  
 Page 307, footnote 5, line 2, "viruliferous (meaning virus-bearing)" should read "viriferous (meaning virus-bearing)."  
 Page 315, line 27, "cheese weed" should read "(cheese weed)."  
 Page 323, line 20, "(Pl. 7, G)" should read "(Pl. 1, F)."  
 Page 323, line 32, "(Pl. 1, C and D)" should read "(Pl. 1, B)."  
 Page 324, line 46, "(Pl. 2, C and D)" should read "(Pl. 2, C and E)."  
 Page 327, line 44, "6 gallons" should read "8 gallons."  
 Page 330, line 2, "of the midrib" should read "on the midrib."  
 Plate 2, legend (page before 377), line 4, "Paragounous" should read "Paragynous."  
 Page 377, line 27, "(Pls. 2, 3, and 4)" should read "(Fig. 1 and Pls. 2 and 3)".  
 Page 423, line 36, "sent away" should read "set away."  
 Page 455, line 7, "The greatest growth of any of the plants measured August 16 was that made during the period of longest days" should read "The greatest growth of any was that measured August 16 made during the period of longest days."  
 Page 532, Table IV, columns 2 and 3, should have a parallel line separating the data of sample 61352 from those of 61375.  
 Page 535, the paragraph immediately following Table V should immediately follow Table III, on page 531.  
 Page 536, Table VI, the headings "Modified Strowd Method" and "Ulsch-Street Method" should read "•Modified Stroud Method" and "•Ulsch-Street Method."  
 Page 542, Table I, column 4, last line (reference to footnote) should read <sup>b</sup>.  
 Page 542, Table II, column 4, footnote reference <sup>a</sup> should be transferred to column 2, line 6, "<sup>a</sup>(Victory, Minn. 514)."  
 Page 545, line 2 of second paragraph, omit "the" before "*phleipratenses*."  
 Legends of Pls. 3, 4, and 5 (following p. 576), capital letters in alphabetic order should replace the numbers 1, 2, 3, etc.  
 Legend of Pl. 4, line 3, should read "K; D, selection I; E probable natural hybrid in selection I.  
 Page 597, paragraph 4, line 2, "Steam one hour; titrate with hydrochloric acid and make up to plus 10, Fuller's scale," should read "Steam one hour, titrate and make up with hydrochloric acid to plus 10 on Fuller scale."  
 Page 668, eleventh line from bottom *Cycticotoichus* should read "Cycticotoichus"  
 Page 668, sixth line from bottom "*Cycticolerachitus*" should read "*Cycticolerachytus*."  
 Page 675, Table 1, line 3, column 7, "7" Bivalent, should read "6".  
 Page 668, line 18, "*Allium cepae*" should read "*Allium cepa*."  
 Page 726, Table IV, lines 4 and 5, "*Atriplex fasciculata*" should read "*Atriplex fasciculata*."  
 Page 731, line 36, "*Eriogonum*" should read "*Eriogonum*."  
 Page 737, line 31, "*Eriodidum*" should read "*Erodium*."  
 Page 767, line 13, "*Baileya pauciradiata*" should read "*Baileya pauciradiata*."  
 Following page 840, legend of Plate 6, line 4, "Gothic-like-shaped" should read U-shaped.  
 Page 849, line 20, " $\Delta = \Delta' - 0.0125\mu\Delta$ " should read " $\Delta = \Delta - 0.0125\mu\Delta$ ."  
 Page 854, in legend to fig. 1, "6.12 inches" should read "6-12 inches."  
 Page 855, in legend to fig. 2, "6.12 inches" should read "6-12 inches."  
 Page 859, Table V, footnote c "atometer" should read "atmmometer."  
 Page 867, Table VIII, line 1, "*Purslua tridentata*" should read "*Purshia tridentata*."  
 Page 902, line 1, "Atkins, W. R. C." should read "Atkins, W. R. G."  
 Page 906, line 1, "Millard, C. E." should read "Millar, C. E."  
 Page 939, Table II, under subhead "1922," column 1, line 8, "September" should read "Do" and line 9, "Do" should read "September."  
 Page 939, Table II, footnote <sup>a</sup>, On Aug. 25, 1922, should read "On Oct. 6, 1922."  
 Page 957, fourth paragraph, line 3, "from 8.6 to 15 mu" should read "from 6 to 15 mu."  
 Page 1042, Table I, last column, last line, the probable error should read .059 instead of .59.  
 Page 1045, in the article "Uninucleated Aecidiospores in *Caeoma Nitens*, and Associated Phenomena," "Plate 5" should read "Plate 4" and "Plate 4" should read "Plate 5," and the legends remain as they are.  
 Page 1061, in the formula, last line " $\gamma$ " should read " $\nu$ ".  
 Page 1063, De Man's formula, " $\gamma - 93.5$ " read " $\mu - 93.5$ ;" and " $\psi - 133.8$ " should read " $\mu - 133.8$ ."  
 Page 1090, bottom line, "in only" should read "is only."  
 Page 1130, third line from bottom, "culture" should read "collection."  
 Page 1165, line 21, "25-50 and 100 mgm." should read "25, 50, and 100 mgm."  
 Page 1215, author's title, "Assistant Chief" should read "Chief."  
 Page 1201, line 26, "1-3 F" should read "1 F-3 F;" and line 26, "Plate 8" should read "Plate 8, C."  
 Page 1215, author's title, "Assistant Chief" should read "Chief."  
 Page 1237, author's emendation: In Table I in the caption "Number of seedlings remaining in 1923, by species and year of origin," insert "per acre" after the word seedlings. In Table II the meaning is confused by the wording of the captions and the use of the word survival. The title of the table should read "Percentages of total number of seedlings present in different years under scattered and under piled and burned slash, all methods of cutting combined." In the first column, the word "survival," used six times, should be replaced by the word "count." In the second and third primary headings the words "of survival" and "by species" should be eliminated. It will be seen that in any column the sum of the figures for scattered and piled and burned slash for a given year is 100 per cent. In other words, the percentage of survival is not being discussed, but the percentage of the total number of each species (100 per cent) present at any count on all of the cut-over plots which occurs on the slash-scattered area and on the piled and burned area.  
 Page 1238, line 5, the words "third year" at the end of the line should be replaced by the words "first three years."

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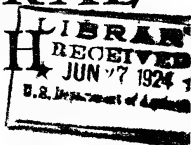
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## CONTENTS

	Page
Anatomy and Metamorphosis of the Apple Maggot, <i>Rhagoletis pomonella</i> Walsh - - - - -	1
R. E. SNODGRASS (Contribution from Bureau of Entomology)	
Chemical and Biological Studies with Cyanamid and Some of Its Transformation Products - - - - -	37
K. D. JACOB, F. E. ALLISON, and J. M. BRAHAM (Contribution from Fixed Nitrogen Research Laboratory)	
Grossularia echinella, a Spiny-Fruited Gooseberry from Florida -	71
FREDERICK V. COVILLE (Contribution from Bureau of Plant Industry)	
Fowl Typhoid, Its Dissemination and Control - - - - -	75
B. F. KAUPP and R. S. DEARSTYNE (Contribution from North Carolina Agricultural Experiment Station)	
Effects of the Modified Hot-Water Treatment on Germination, Growth, and Yield of Wheat - - - - -	79
V. F. TAPKE (Contribution from Bureau of Plant Industry)	

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No. 1

## ANATOMY AND METAMORPHOSIS OF THE APPLE MAGGOT, RHAGOLETIS POMONELLA WALSH<sup>1</sup>

By R. E. SNODGRASS

*Deciduous Fruit Insect Investigations, Bureau of Entomology, United States Department of Agriculture*

### INTRODUCTION

Since all things of the present are products of the past, we can understand nothing rightly without knowing its history. This applies even to insects. But, among insects, so many species are so much alike that it is not necessary to know all phases of each one separately; the story of one may be repeated in the history of many others. Thus, the study of the apple maggot, here presented, explains a type of development and structure common to a large group of flies that includes the various other fruit-flies, the root-maggots, the house fly, the blow-fly, the sheep tick, and all the host of forms related to these species, embracing most of the important fly pests of man, domestic animals, and cultivated plants, as well as many beneficial species that are parasites of other insects.

The larval form of these flies is called a maggot, but the maggot has had the strangest history of all insects, and an obscure one, too, for it is known only to a few entomologists. Yet, because of the economic importance of maggots, much has been written about them and, unfortunately, many of the published descriptions are vague or inaccurate because the writers did not understand the history of their subjects.

The apple maggot, the larva of *Rhagoletis pomonella* Walsh, is an insect highly favored by nature. It lives among a superabundance of food, it grows to maturity in almost complete security from enemies, and is never exposed to the chance of being poisoned by the sprays of the orchardist. Its mother is a small fly (fig. 1, A) with a sharp drill at the end of her body by means of which she pierces the skin of the apple (fig. 1, B) and deposits her egg in the flesh beneath. The young maggot, hatching from the egg, has only to burrow into the fruit (fig. 1, C), for here it spends its days tunnelling about and feeding on the pulp (fig. 2) until it is full grown.

### EXTERNAL STRUCTURE

In general appearance the apple maggot is a smooth, white, wormlike creature, 7 or 8 mm. in length when full-grown, having the external form and structure shown in Plate 1, A. Its body consists of eleven distinct segments and a small, retractile, conical end-piece in front (*LH*), which serves as a head and carries the pair of mouth hooks (*Hk*). The first body segment (*I*) bears the anterior larval spiracles (*ASp*) and the last segment (*VIII*) carries the posterior spiracles (*PSP*) and the anus (*An*).

<sup>1</sup> Received for publication February 21, 1924.

There has been much useless discussion in print on the subject of the number of segments in the maggot, by writers unfamiliar with the fundamental facts of its structure, though the correct morphology of the muscoid larva has been known for nearly 60 years, ever since Weismann (53)<sup>2</sup> published the results of his studies on the blow-fly. That the segment carrying the anterior spiracles (Pl. 1, A, *ASp*) is the true first body segment is proved by the fact that the stalks

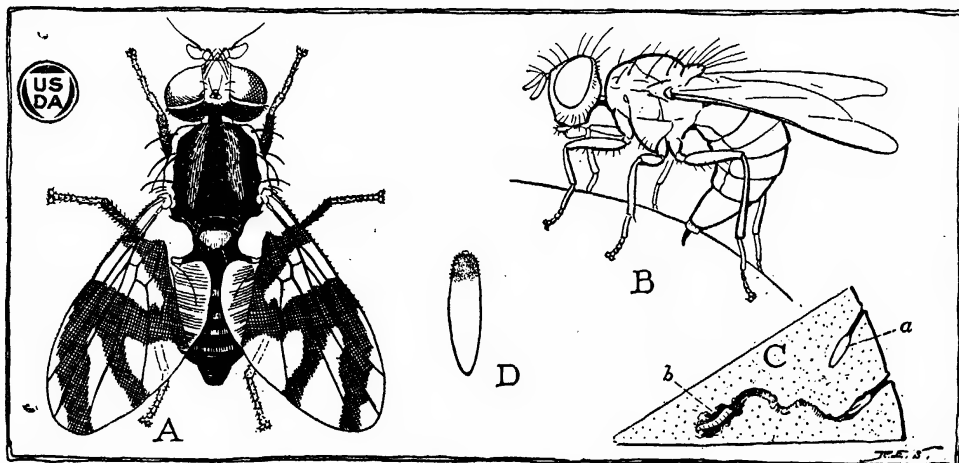


FIG. 1.—The apple maggot (*Rhagoletis pomonella*): A, adult female ( $\times 7$ ); B, female fly puncturing the skin of an apple preparatory to depositing an egg; C, section of an apple showing an egg inserted at *a*, and a young maggot tunnelling into the pulp at *b*; D, an egg (greatly enlarged)

of the prothoracic leg buds are attached to its ventral surface (Pl. 3, F, *L*<sub>1</sub>) and evaginate from it. Likewise the attachment of the other leg buds (Pl. 3, F, *L*<sub>2</sub>, *L*<sub>3</sub>) and the buds of the wings and halteres to the next two segments proves that these (2 and 3) are the mesothorax and the metathorax. The following eight segments (*I–VIII*) belong to the abdomen, but the last one is evidently a combination of the primitive eighth, ninth, and tenth segments.

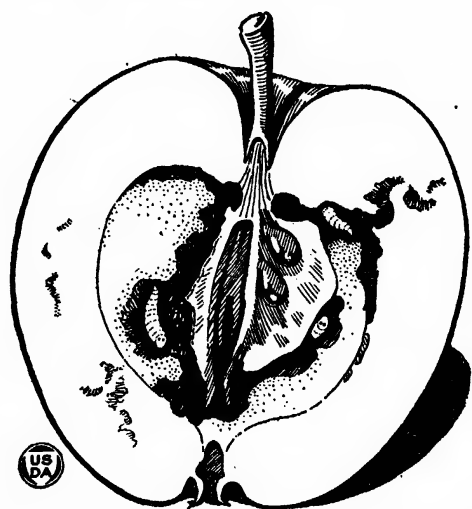


FIG. 2.—An infested apple cut open showing the tunnels of the apple maggot through the flesh

The larval head in some species of maggots is divided into several parts by transverse constrictions, one of which has been called Newport's "segment," but such rings are not true segments. Moreover, a study of the maggot's development shows that its apparent head is, at most, but a small part of the true head, and that the mouth hooks are not "mandibles" as some writers assume them to be.

The larval head (Pl. 1, A, *LH*) of the apple maggot is a simple cone, sometimes suggesting a double structure by a transverse fold which appears when the head is partially retracted. The anterior end is blunt and snoutlike (Pl. 1, B) and bears two papillæ (*g*, *h*) on each of its anterior angles. These papillæ support peglike sensory end-organs, shown more enlarged in Plate 1, C and D. On the middle of the lower surface of the head is the larval mouth (Pl. 1, B, *Mth*) from which project the two large oral hooks (*Hk*). These hooks lie in lateral pouches of the oral cavity formed by median dorsal and ventral tonguelike ridges (*k*

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 33–36.



and *l*). On the lateral lips of the mouth are two pairs of chitinous teeth which appear to serve as guards against the hooks. Immediately laterad of each pair of hooks is a third sensory organ (*i*), shown more enlarged in Plate 1, E. The lips of the mouth, posterior to the teeth, are marked by ridges as shown in Plate 1, B. In some other species these ridges are more extensive and spread out like the ribs of a fan from the angles of the mouth. They perhaps serve to conduct the liquid food of the maggot into the oral cavity.

The oral hooks (Pl. 1, A, B, *Hk*) are strong organs of the mouth formed for grasping, clawing, and tearing. The apple maggot uses them for tunnelling through the fruit and for breaking down the pulp in order to liberate the juices and protoplasm of the apple cells which constitute its food. The hooks move up and down on a basal articulation, not sidewise as do mandibles, and they can be protruded or withdrawn by an evagination or retraction of the oral cavity. When the hooks are very forcibly withdrawn the head itself frequently disappears by inversion into the thorax. The mouth hooks differ characteristically in the three larval stages as shown in Plate 1, L, M, and N. In the first instar (Pl. 1, L) the second or proximal claw is well developed, in the second instar (Pl. 1, M) this claw becomes reduced, and in the third (Pl. 1, N) it disappears. The hooks are articulated to a chitinous framework in the wall of the pharynx and are worked by sets of special muscles, but those parts will be described in connection with the pharyngeal skeleton.

On the body of the maggot the intersegmental lines are marked by circular swellings (Pl. 1, A) widest on the ventral surface and most pronounced on the posterior two-thirds. All but the first of these ridges are covered by minute hooklets (Pl. 1, G) arranged in several irregular, broken rows, forming bands at the junctures of the segments. In the wider bands the hooklets of several extreme anterior and posterior rows are turned forward and backward respectively (Pl. 1, F), while those of the median rows are turned backward and forward respectively. That the band of hooklets does not mark the end of either of the adjoining segments is shown by the attachment of the muscles of the body wall (Pl. 4, C, E) along distinct lines between the median rows of hooklets in each band.

Anterior spiracles are present only in the second and third instars of the larva, and in the pupa, and they differ in each stage, since each successive pair is newly formed and not developed from those of the preceding instar. But the spiracles are always located on the prothorax and, in the larva, project like ears from the upper lateral parts of this segment close to the posterior margin (Pl. 1, A, *ASp*). Each is a lobelike structure, flattened in a vertical plane, arising from a small mound of the hypoderm. The free edge carries many small papillae. The spiracle of the second instar (Pl. 1, H) is smaller and simpler than that of the third. In the third instar the lobe is somewhat subdivided and the papillae are longer (Pl. 1, J). The papillae are perforated at their ends, though ordinarily it is impossible to see the openings. But if a larva is killed in hot water and transferred immediately to cold water or to weak alcohol, the ends of the papillae will be turgid with air, and a high-power objective will often show a slitlike aperture gaping wide in the tip of each (Pl. 1, K). Pressure on the base of the lobe will discharge tiny bubbles of air from these apertures.

The openings of the spiracle lead into a cavity within the lobe, which is prolonged backward like a stalk beneath the hypoderm to meet the end of one of the two great dorsal trunks of the tracheal system (Pl. 2, G, *Tra*). This cavity is the *stigmatic chamber* (Pl. 1, H, J, *SpC*). It is always distinguished from the adjoining trachea by its yellow color and the granular texture of its walls, and by the lack of spiral thickenings in its lining. In the second instar the stigmatic chamber of the anterior spiracle is a simple slender tube (Pl. 1, H, *SpC*); in the

third instar it is much larger and presents a clear, circular, windowlike area (Pl. 1, J, *n*) in the outer side of its base, directed outward and forward. This area is connected with the body wall (Pl. 1, I, *BW*) just ectad of the base of the external lobe by a short cuticular tube (*v*) which ends in a circular scar (*m*) on the body wall. The same structure is repeated in the prothoracic spiracle of the pupa (Pl. 2, A) and we shall see that it is characteristic also of the posterior spiracles of the second and third larval instars. It is to be explained as follows: Each spiracle is a newly formed structure developed, not within that of the preceding instar, as is usual in other cuticular structures, but from the inner side of the base of the stigmatic chamber. At each molt the old spiracle is cast off and the lining of the stigmatic chamber, together with the tracheal intima, is drawn out attached to it through the channel formed by the new cuticle of the old chamber. The latter then shrinks, as the new chamber becomes functional, and is reduced to the cuticular tube (*v*) connecting the base of the chamber with the new chitinous layer of the body wall, the closed ends forming the external and internal scars (*m* and *n*).

The successive formation of new spiracles in each instar of the fly larva has been described by De Meijere (30) for such a large series of genera from *Mycetophila* to *Hydromyza* that there can be no doubt that it is characteristic of all the amphipneustic Diptera. Though the form of the external spiracular lobe may vary from a simple projection to a many-fingered flap or to a long, slender tube, the fundamental structure is always the same. It has not been proved for all stages that the new stigmatic chamber is an outgrowth from the old, but Weismann (54) has shown in *Corethra*, and Wahl (49) in *Eristalis*, that the stigmatic chambers of the pupal spiracles grow out from the ends of the tracheæ as solid masses of cells, proliferated from the tracheal epithelium, in which a lumen appears later. It would seem more reasonable that the chambers should be formed as secondary ingrowth from the body wall since they have no tænidia nor any other appearance of tracheal structure, and Haake (13) says that they are thus formed in the larva of *Trigona*. De Meijere (30) says that the inner walls of the chamber ("filzkammer") are usually ciliated, and that in some forms they are braced by chitinous bars across the cavity. In *Rhagoletis* the chambers are unobstructed and their walls appear to be rugose but not ciliated.

These dorsal spiracles of both the larva and the pupa are, in any case, secondary structures. They are developed in connection with the *dorsal* longitudinal tracheal trunks (Pl. 2, G), while the primitive spiracles, temporarily closed in the maggot, belong to the *lower* lateral trunk. That special breathing structures of a similar nature occur in other orders is indicated by the description of a respiratory organ in a beetle larva, *Donacia*, given by Böving (9). Here a hollow, apparently perforate, spinelike process is situated on the back of the eighth segment over an end-chamber of a trachea, and contains a prolongation of the latter. At each molt the spine is cast off, bringing the tracheal intima with it, while a new spine is generated by the new cuticle and a new branch of the tracheal end-chamber grows out into it.

The last body segment of the maggot is the largest (Pl. 1, A, *VIII*). The posterior part of its upper surface is flat, slopes downward, and bears the posterior spiracles (*PSp*). The anus (*An*) is situated on the rear part of its ventral surface between a pair of lobes. The stigmatic surface is surrounded by eight tubercles, two above and two below the spiracles, and two very small ones on each side. A fifth pair occurs between the substigmatic pair and the anal lobes. In the pupa the eighth pair of abdominal spiracles is situated on this segment, and the part bearing the anus is differentiated as a small segment which becomes the tenth of the adult. The terminal segment of the larva, therefore, is evidently a combination of the eighth, ninth, and tenth abdominal segments.

The posterior larval spiracles (Pl. 1, A, *PSp*) are present in each of the larval instars. In the first instar each spiracle has two apertures (Pl. 2, D), in the second (Pl. 2, B) and third (Pl. 2, C) three apertures. The openings are surrounded by dark chitinous rims known as the *stigmatic plates* (Pl. 2, D, *SpPl*), which have different shapes characteristic of each instar. Beneath the plates are stigmatic chambers (*SpC*) set on the ends of the tracheæ, which are similar in structure to those of the anterior spiracles and have the same yellow color.

The stigmatic plates of the posterior spiracles of the first-instar larva are simple oval rims about each aperture, widest on the outer sides (Pl. 2, D, *SpPl*). Four groups of delicate radiating hairs arise from the cuticle at the upper, outer, and lower margins of each pair of plates. The stigmatic chambers (*SpC*) are long, tubular end-sacs of the tracheæ (*Tra*), in line with the tracheal axes.

In the second instar the posterior stigmatic plates are elongate-oval in form and each has from six to eight teeth on the inner edge of each lip guarding the aperture to the stigmatic chamber (Pl. 2, B). Four large, fan-shaped bunches of hairs arise from the cuticle around each spiracle, one above, one below, and two lateral between the outer ends of the plates. Each stigmatic chamber (*SpC*) divides distally into three lobes corresponding with the three stigmatic plates and their apertures. On the inner side of its base is a small circular scar connected with the body wall by a shrunken strand of cuticle (*v*). This, as in the case of the anterior spiracles, is the remnant of the channel through which the first stigmatic chamber and the lining of the tracheæ were drawn out at the first molt. The position of the new spiracles, however, is reversed at the two ends of the body; in front the new spiracle being formed mesad to the old.

In the third instar the apertures of the posterior spiracles are linear slits in long, slender stigmatic plates (Pl. 2, C). Each plate is a cylindrical chitinous structure at the end of a wide lobe of the stigmatic chamber, and is open to the exterior the full length of its outer surface and at each end (Pl. 2, E). The lips of the opening are fringed with many slender prongs that curve inward like the teeth of a pair of rakes turned toward each other. The structure of these spiracles is particularly conspicuous when a larva is submerged in water, for the air in the spiracles may then be seen glistening like mercury between the prongs of the slits of each plate and bulging out at the unguarded ends. Four radiating groups of hairs arise from the cuticle about the stigmatic plates, as in the second instar, but in this, the third instar, most of the hairs are branched. The stigmatic chambers (Pl. 2, C, *SpC*) are much larger than in either of the other instars, and the scars in their bases, left by the chambers of the second instar, are large and conspicuous. These spiracles do not reappear on the pupa. When the stigmatic chambers and tracheal linings are cast out at the last larval molt the holes in the body of the pupa close.

### THE TRACHEAL SYSTEM

The most conspicuous features of the larval tracheal system are the two large dorsal trunks that extend between the anterior and the posterior spiracles (Pl. 2, G, *Tra*). The trunks are connected by a dorsal commissure in each segment but the first, and they give off 11 descending lateral branches. The first and the last dorsal commissures (*q* and *s*) are much larger than the others and go straight across from one longitudinal trunk to the other. The rest (*r*) are very slender tubes and each makes a long V-shaped loop into the preceding segment. The lateral branches from the dorsal trunks (Pl. 2, F) supply tracheæ to the viscera and to the body walls. All but the first two are connected below the middle of the sides by a series of longitudinal commissures (*t*). These commissures are seldom shown in drawings of the tracheæ of the maggot, but they are a very important feature of the general tracheal system; they constitute, in fact, the

ventral lateral trunk which is always connected with the primitive spiracles. In the maggot the lateral spiracles are undeveloped and the lateral tracheal trunks are reduced in size till, by comparison with the highly developed dorsal trunks, they become inconspicuous and appear to be mere commissures between the descending branches from the dorsal trunks. But the lateral spiracles appear on the puparium (Pl. 5, A) and are opened in the pupal stage by the shedding of the tracheal linings. Finally they become functional in the adult, where the lower lateral tracheal trunk regains its importance and the dorsal one is reduced again to usual size.

Perhaps the lateral spiracles are present in some form in the hypoderm of the larva, but it is impossible to see them by surface examination until the skin hardens to form the puparium. Van Rees (47) noted in *Calliphora* that the lateral stigmatic tracheæ do not penetrate the hypoderm in the larva or in the puparium, but that each ends in a small island of cells which forms a part of the outer surface of the body. These islands regenerate the epithelium of the stigmatic tracheæ and that of neighboring parts of the longitudinal trunks. Wahl (50) regards them as true hypodermal imaginal buds and says that they are present on the metathorax and on the first seven abdominal segments in *Eristalis*, as noted also by Giacomini (12). Van Rees (47) finds them on all the abdominal segments but the eighth in *Calliphora*. Pratt (40) says that there is a series of eight microscopic, rudimentary stigmata on young larvæ of *Melophagus ovinus* L., two on the thorax and six on the abdomen. In the puparium of *Rhagoletis* (Pl. 5, A) eight pairs of spiracles appear on the abdominal segments, but the last is very small.

It is thus evident that the two sets of spiracles in the Diptera have no relation to each other. The temporary, dorsal larval spiracles are developed in connection with the dorsal longitudinal tracheal trunks; the primitive spiracles belong to the lower lateral trunks. The fundamental tracheal system is the same in both larva and adult, but the longitudinal trunks are proportionately developed in each stage according to the spiracular system in use—the larva and pupa using the dorsal system, the adult using the ventral system.

The larvæ of the lower flies are peripneustic like the adults, and the presence of the stigmatic islands of Van Rees in the hypoderm of the larvæ of higher forms shows that even in them the lateral spiracles have not been entirely lost. Their final opening is but a restoration to primitive conditions. The tracheal system of a cecidomyiid fly larva (*Cecidomyia resinicoloides* Williams) as described and figured by Williams (55) consists of two large dorsal trunks extending between the anterior and the posterior spiracles, and of two slender lateral trunks connected with lateral spiracles. The anterior spiracles are on the prothorax; the posterior spiracles are on the last abdominal segment, which is the ninth. The lateral spiracles occur only on the first seven abdominal segments. The condition here strongly suggests that both respiratory systems are present and functional at the same time in this larva, and that the lateral spiracles are in an early stage of repression. The spiracle tracheæ are still well developed in the metathorax, though the spiracles of this segment are lacking.

The larva of the higher flies, then, temporarily discards the primitive lateral spiracles and develops breathing organs of its own in connection with the dorsal tracheal trunks. It acquires first a pair of spiracles at the rear end of its body, and is metapneustic in its earliest instar. Next it acquires a second pair at the anterior end of the body, and is amphipneustic in the second and third instars. The pupa retains only the anterior spiracles of the larval system, and is proneustic. The adult discards all the larval spiracles, restores the primitive breathing system of its ancestors, and is again peripneustic.

In the facts thus outlined a basis might be found for believing that the fly maggot owes its form and structure to an early but secondary aquatic life when it floated half submerged on the surface of water. In such an environment the enlarged dorsal tracheal trunks would serve as floats, the dorsal spiracles would facilitate breathing above the surface, and the closing of the lower spiracles, with the reduction of the lateral tracheal trunks, would follow as logical modifications. The maggot form is probably not necessary, or the only one possible, in the present environments of most muscoid larvæ, but certainly, once acquired, it is one well adapted to many conditions of living and gives the muscoids a wide range of possible environment with few modifications of structure.

### THE IMAGINAL BUDS

The larva of insects with complete metamorphosis consists of two sets of cells, one set including all of those cells that constitute the larval organs, the other those that will form the adult organs which replace larval parts. In general, the adult cells are much smaller than the cells of the larva, and, in the larva, they are centered in small groups called *imaginal buds*, *imaginal discs*, or *histoblasts*. It is now pretty well established for the Diptera that there are four imaginal buds in each segment, and that, all together, they form four rows of discs in the hypoderm, two in line with the wings and two in line with the legs. This applies theoretically also to the head, but the condensation of the segments in this region obscures the primitive arrangement of the buds. Each bud consists of both ectodermal and mesodermal cells, the first regenerating the hypoderm and all parts derived from it, the second regenerating new muscles and perhaps other mesodermal tissues. The alimentary canal is reformed from special cells situated in its own walls.

Most of the histoblasts do not begin their regenerative growth until the last larval instar. But, in the case of appendages that have disappeared entirely in the larva, the histoblasts may take an early start, and, in the higher Diptera, some of these begin their development in the embryo. Such buds must necessarily push inwardly in their growth, and, as a consequence, they come to lie in pockets of the hypoderm, called *peripodal cavities*. In the muscoid larvæ these pockets of the leg buds form long, stalked pouches entirely closed at their outer ends (Pl. 3, F,  $L_1$ ,  $L_2$ ,  $L_3$ ). All such organs that begin in this precocious manner must later be everted in order to complete their development. This takes place in the pupal stage.

The saclike buds of the legs and wings growing inside the body were observed in many insects long before their true nature was known. Weismann (53) showed that they evert to form the external appendages and gave them the name of "imaginal discs." In Corethra he noted (54) that they arise from the hypoderm, but he failed to see this in Calliphora.

Künckel d'Herculais (27) first demonstrated that the buds of external parts are always of ectodermal origin. He proposed the name "histoblast" as a term more generally appropriate than Weismann's "imaginal discs." He, moreover, enumerated 12 pairs of buds in the Syrphidae—4 pairs in the head, 6 pairs on the thorax, and 2 on the abdomen which form the genital armature.

Ganin (11) added the fact that there are two pairs of buds on each of the abdominal segments, and that these buds regenerate the abdominal hypoderm and its appendages wherever appendages occur. He showed also that each bud consists of both hypoderm and mesoderm.

Viallanes (48) likewise recorded the presence of four buds on each of the abdominal segments, and pointed out that these fall in line with those of the thorax, forming thus four rows of buds along the entire length of the body in the larva that regenerate the external parts of the adult. Kowalevsky (25), studying Muscoid larvæ, found a dorsal and a ventral pair of buds on each of the first

seven segments of the abdomen and corresponding pairs on the eighth surrounding the anus. These abdominal buds, he says, are mere islands of small cells among the larger cells of the larval hypoderm. They regenerate the new hypoderm of the pupa, and those of the last segment form also the new epithelium of the rectum and the rectal papillæ.

Van Rees (47) describes three pairs of buds on each of the abdominal segments in *Calliphora*, two dorsal and one ventral. The second dorsal buds, he says, are very small, however, and lie close to the others with which they soon unite when they begin to expand. Wahl (50) finds also three pairs on the abdominal segments of *Eristalis*, two dorsal and one ventral, but the two dorsal pairs widely separated and situated close to the opposite edges of the segment. This is apparently an exceptional condition. Giacomini (12) describes only two pairs of buds on each of the abdominal segments of *Eristalis tenax* L. and says that they have the form of small invaginations in the mature larva.

Pratt (40), discussing imaginal buds of all insects with complete metamorphosis, sees so much significance in the presence of four rows of them that he concludes that the ancestors of insects had four series of segmental appendages along the entire length of the body. Such a theory is unwarranted if we regard the buds as the regenerative centers of each segment as a whole, and not as the histoblasts, primarily, of an appendage. The presence or absence of a leg or a wing is incidental, though the invaginated form of the bud is due to the appendage which the bud is to form. Yet all appendage-forming buds are not invaginated, as has been shown by Needham (36) for some beetles, where the bud is a flat disc of cells which grows outward to form the appendage beneath the larval skin.

This discussion would be more in place in the section on metamorphosis were it not for the fact that an understanding of the imaginal buds in general is necessary for an understanding of the morphology of the head and pharynx of the maggot. The metamorphosis of the higher flies *begins in the embryo*. Normally metamorphosis has to do with the transformation of the larva into the adult, and, in its more primitive manifestations, the changes do not begin until the end of the last larval stage. In the *Diptera*, however, the reconstructive growth has started at successively earlier and earlier stages during evolution, till, in the higher forms, some of the imaginal parts now begin to develop in the embryo.

### THE LARVAL PHARYNX AND THE FRONTAL SACS

The mouth of the larva opens into a large antechamber of the alimentary canal which is functionally the larval pharynx. An elaborate chitinous skeleton (Pl. 3, B) is developed in its walls and forms the dark chitinous structures that show through the skin in the anterior part of the maggot's body, and which support the oral hooks and their muscles. Most of the illustrations published in systematic and economic papers on Muscoid larvæ show only the parts of the pharyngeal skeleton that are chitinized strongly enough to be seen through the overlying muscles and body wall. Since such figures do not give the true form or structure of the plates, they have no morphological value and convey no idea of the functions of the parts.

An intelligible description of the pharynx and its skeleton must be based on a clear understanding of two well known facts in the development of the head of the maggot and of the adult fly. The first is that the head of the fly is formed largely from two sacs inverted deeply into the body from the frontal region of the larval head. The second is that, in the higher *Diptera*, the true head of the larva has been invaginated, not retracted, into the mouth and has carried with it the two frontal sacs and their points of origin in the hypoderm. Hence, the so-called head of the larva is really only the neck and possibly a small part of the



base of the true head. Most of the head forms the larval mouth cavity and a part of the larval pharynx.

An examination of figure 3 will show in a very simple, diagrammatic way just what has taken place in the evolution of the maggot's head and how the parts have come to be as they are. The head of the adult fly is formed by a restoration of the invaginated parts to their normal positions, and by the development of the mouth appendages from buds in their proper morphological places. The reader should note here that the usual diagrams of the metamorphosis of the muscoid head, taken from Korschelt and Heider (22), are incorrect in that they represent the frontal sacs as arising from the rear end of the larval pharynx and not from its anterior end. Such a scheme leaves nothing to account for the anterior parts of the head invaginated in advance of the basis of the frontal sacs.

The structure of the pharynx and its appendages in the larva of *Rhagoletis* is shown in Plate 3, C. The pharynx itself is a wide passageway from the mouth,

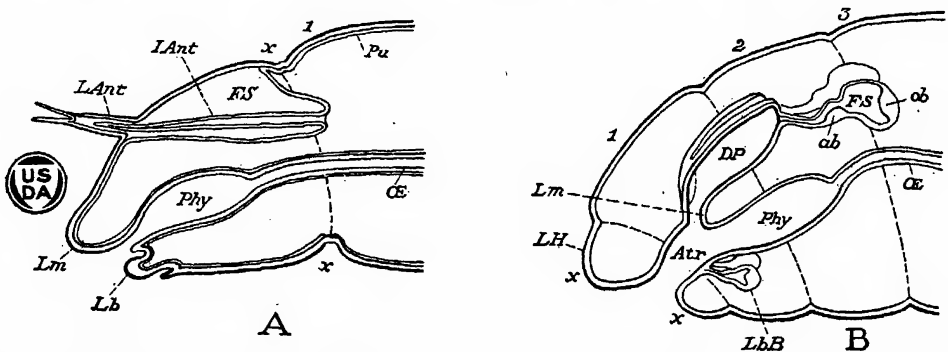


FIG. 3.—Showing the changes that have taken place in the head of a fly larva during evolution from the primitive condition of lower forms (A) to the complex structure of higher forms (B). A, diagrammatic lengthwise section of head and prothorax of larva of a lower fly, such as *Chironomus*, with external mouth parts (*Lm*, *Lb*) and with a small larval antenna (*LAnt*), but with a long imaginal antenna (*IAnt*) growing within a frontal sac (*FS*) or deep invagination of the hypoderm of the forming pupa (*Pu*) and with only its tip in the larval antenna; the pharynx (*Phy*) and oesophagus in normal position. B, a corresponding section through head and thorax of the maggot of a higher fly. The larval appendages are gone and the head has been turned in to form a new cavity, the atrium (*Atr*) in front of the pharynx (*Phy*), with a secondary evagination from its roof forming the bilobed dorsal pouch (*DP*) carrying the frontal sacs (*FS*) at the inner ends of its wings. The frontal sacs contain the imaginal rudiments or buds of the antennæ (*ab*) and the compound eyes (*ob*). The labrum (*Lm*) is now buried behind the root of the dorsal pouch of the atrium, and the buds of the imaginal labium (*LbB*) arise beneath the floor of the atrium. The lips of the new mouth (*x, x*) are derived from the back of the original head (*A, xx*) and the larval head (*LH*) must be the primitive neck region. (Cf. Pl. 3, F.)

between the oral hooks (*Hk*), to the oesophagus (*OE*). Its floor (*VPhy*) is a broad, convex membrane having a ribbed appearance externally (*d*) due to lengthwise ridges on its inner surface (Pl. 3, D, *d*). The lateral walls are strengthened on each side by two plates, a small triangular one (Pl. 3, C, *A*) in front and a much larger one (*B*) behind. The first plate supports the oral hook of the same side by a loose articulation at its anterior angle. Its posterior angle is more or less fused with the second plate, and its ventral angle is connected by a transverse bridge (*e*) in the floor of the pharynx with the corresponding plate of the opposite side (Pl. 3, E, *e*). A small rod from its upper edge (Pl. 3, C, *a*) helps support the roof of the pharynx, which is further strengthened by a bridge plate (*D*) between the anterior ends of the triangular plates (*A*).

The anterior part of the pharyngeal roof is convex and covers an anterior section of the pharyngeal cavity called the atrium by Wahl (51), shown diagrammatically (*Atr*) in figure 3, B. From the posterior end of the atrium the roof of the pharynx is produced upward as a dorsal pouch (Pl. 3, C, *DP*) having a

semicircular base reaching back on the sides through the first third of the lateral plates (*B*). This pouch soon divides into two wings which are produced backward nearly as far as the end of the pharynx, and are then prolonged into two long, stalked sacs (Pl. 3, A, E; fig. 3, B, *FS*). The inner surfaces of the outer walls of the two divisions of the dorsal pouch are chitinized and form the two dorsal wing-plates (Pl. 3, C, *C*) of the pharyngeal skeleton. These wings are supported by wide stalks from the upper edges of the posterior lateral plates (*B*), developed in the lateral parts of the semicircular base of the dorsal pouch (*DP*), and they are united with each other dorsally at their anterior ends by a narrow bridge in the upper part of the basal undivided part of the pouch. The stalked bags (*FS*) continued backward from the ends of the pouches are the *frontal sacs* which contain the buds of the imaginal antennæ and compound eyes.

Plate 3, D, shows a transverse section of the pharynx through the stalks of the wing plates. The roof of the pharynx is deeply concave behind the base of the dorsal pouch as here shown (*DPhy*) and as indicated in Plate 3, C. The space between the roof and the wing plates is filled with the dilator muscles of the pharynx (Pl. 3, A, D, *DiMcl*), but these will be described later.

The earlier phases of the evolution of the larval and imaginal head of the Diptera have been shown to exist in the larvæ of Culicidæ and Chironomidæ by Weismann (54) and by Miall and Hammond (33). The latter writers state that the invaginations that are to form the head of the fly in *Chironomus* do not begin to form, even in a rudimentary state, until after the last larval molt. Then they appear as simple infoldings along the edges of the frontal plate of the larval head. Later they unite posteriorly and the wide single pouch resulting is produced posteriorly into the thorax. In this pouch are developed the antennæ, the compound eyes, and the surrounding head region of the adult. The eversion of the parts can be observed when the larva changes to a pupa. According to Miall and Hammond (34) the earliest developmental stage of the frontal sacs in *Chironomus* represents the maximum attained in *Corethra*, while the sacs are still more primitive in a mosquito larva.

In the Muscoidea and Hippoboscidae the frontal sacs reach their highest development, but Pratt (41) has shown that in *Melophagus* they still originate in a pair of simple thickenings in the hypoderm on the dorsal wall of the head in the embryo. In *Eristalis* and *Calliphora* as shown by Wahl (49, 51), the sacs arise from a single median bud, but it seems probable that an earlier paired stage is here eliminated, for the two rudiments in *Melophagus* soon unite.

The paper by Pratt (41) on the history of the imaginal buds in *Melophagus ovinus* is the most important work we have on the embryology of the larval head in the Diptera. Our knowledge of the subject began with the work of Weismann (53, 54), who discovered that the head of the larva is almost completely invaginated into the body, and that the head of the adult is formed by an eversion of concealed parts. But Weismann was mistaken in believing that the sacs in which the adult head is formed are produced by the involution of the larval head. He did not distinguish the two processes, already described in this paper, namely, the formation of the frontal sacs containing the imaginal buds of the antennæ and eyes, and the withdrawing of these sacs into the body by the second process of the involution of the larval head.

Pratt (41) shows that the cephalic buds of *Melophagus* appear at a very early stage in the embryo, before the lateral mesodermic bands have extended to the dorsal side, and before the endoderm has enveloped the yolk on the ventral side. They consist at first of a pair of simple thickenings of the hypoderm on the external dorsal surface of the head just behind the stomodeum. The latter is dorsal at this stage but soon moves forward, followed by the buds, which now begin to

invaginate, forming deep crescentic slits immediately ectad to the rudiments of the cerebral ganglia. Next, the slits approach the midline where their outer ends unite to form a single, median slit, while their inner ends remain separate and increase much in size and depth. Thus is established the Y-shaped form, which is retained with subsequent modifications throughout the larval life. This stage is shown in figure 4, A, where only the unpaired basal part of the frontal sacs (*FS*) appears in a median section.

After the union of the frontal sacs in *Melophagus*, according to Pratt's account, the involution of the head takes place. The lips of the mouth are rolled inward, closely followed by the common base of the frontal sacs which are thus carried forward, downward, and finally posteriorly into the new mouth along with the walls of the head (fig. 4, B). There is thus established not only a new mouth, but also a new section of the digestive tract. The latter becomes the anterior part or atrium (*Atr*) of the larval pharynx. The frontal sacs (*FS*), now arising from the rear part of the newly established atrium, grow rapidly and soon attain the form they have in the young larva.

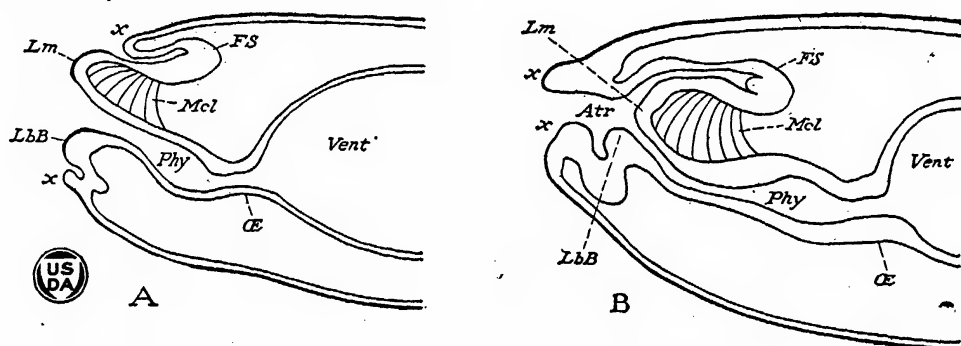


FIG. 4.—Diagrams of the development of the larval head parts in *Melophagus ovinus* (from Pratt, but relettered with parts omitted). A, a young embryo with head still in normal position, the frontal sac (*FS*) opening on frontal surface behind the labrum (*Lm*). *FS*, frontal sac; *LbB*, labial bud; *Lm*, labrum; *Mcl*, labial muscles; *O*, oesophagus; *Phy*, pharynx; *Vent*, ventriculus; *x, x* external fold of hypoderm at base of head. B, an older embryo after the involution of the head. The new mouth is established by the folds *x, x*, the atrium (*Atr*) by the walls of the head invaginated in front of the folds, carrying to the interior the frontal sac *FS* and the labial buds (*LbB*) and burying the labrum (*Lm*) and its muscles (*Mcl*) deep in the body. The labral muscles thus become the dilators of the pharynx. Comparing these figures with those of figure 3 shows a complete parallelism between this individual embryonic development and the evident phylogenetic modification of the dipteran maggot

The more recent paper by Wahl (51) on the formation of the head in the cyclorrhaphous Diptera contains an exhaustive account of the head development as it takes place in *Calliphora*. In the blow-fly and in other species studied by Wahl (*Eristalis*, *Allium*) the frontal sacs originate in a single median evagination from the anterior part of the larval pharynx, forming a suprapharyngeal cavity, from which the two sacs extend backward into the thorax. Probably an earlier stage that would show the separate origin of the cephalic sacs is omitted in these species.

Pratt believed that the larval pharynx (fig. 4, B, *Phy*) results from the invagination of the larval head. Wahl showed that the larval head forms only the anterior part of the pharynx, the part which he calls the *head atrium* and from which arises the common base of the frontal sacs (*FS*).

It would seem that there must be some morphological difference between the dorsal pouch of the atrium (fig. 3, B, *DP*), in the branches of which are developed the wing plates of the pharyngeal skeleton (Pl. 3, C), and the true frontal sacs (*FS*), which contain the buds of the imaginal eyes and antennæ. The chitinous wing plates of the pharynx are shed, as are all other cuticular parts, with each

molt of the larva, but the linings of the frontal sacs are not affected by the molts any more than are the linings of the peripodal sacs of the leg and wing buds. This indicates that the dorsal pouch of the atrium is a secondary invagination, and that its wings are not parts of the true frontal sacs which contain the buds of the antennæ and eyes. This idea is suggested also by Pratt's finding that the median root of the sacs is formed after the invagination of the original buds. Both the median slit and the subsequent prolongations from it that carry the original bud sacs inward are apparently, then, to be regarded as the rudiments of the dorsal pouch of the atrium in which the wing plates of the larval pharynx are later developed.

The evolution of the frontal sacs and the involution of the larval head may be traced through the various groups of the Diptera. In the simplest forms, such as *Simulium* and *Bibiocephala*, studied by Kellogg (19), the head and mouth parts of the pupa are formed exactly within the corresponding parts of the larva, except that the larger antennæ of the pupa are forced to back up under the larval cuticle to find space for their growth. In *Corethra*, as described by Weismann (54), the pupal antennæ are developed in deeply invaginated pouches, but the compound eyes are formed flush with the surface, immediately beneath the larval cuticle. In *Chironomus*, as shown by Miall and Hammond (34), both the antennæ and the compound eyes are developed in pouches formed by infoldings along the sides of the front. This gives the first example of a well-formed frontal sac in the *Nematocera* (fig. 3, A).

In the *Brachycera*, as described by Becker (3) and mentioned by Wahl (51), *Stratiomys* and *Antherix* have frontal sacs which open on the upper, external surface of the head, in *Stratiomys* even well back from the anterior end. Likewise, in *Lonchoptera*, as described by De Meijere (31), the frontal sac opens on the outside of the head, there being no atrium present. These brachycerous forms, therefore, present the same condition of head development as is found in the *Nematocera*, and De Meijere designates them as the "Anatria." In *Corethra* (Weismann) and *Chironomus* (Miall and Hammond) the imaginal buds, including those of the head, are not formed till the last larval stage. The time of their origin in other *Orthorrhapha* is not known.

It is only in the *Cyclorrhapha*, so far as known at present, that the frontal sacs become drawn into the interior of the body by the involution of the larval head, with the consequent formation of a new anterior section of the larval pharynx, the head atrium. Such forms are the "Atriata" of De Meijere. In the higher families of this group (*Muscidae*, *Hippoboscidae*) all the imaginal buds begin their development in the embryo, suggesting that the high degree of development they have attained has forced them to this precocious start.

Thus we see that, as complicated as the head structure may be in the larvæ of the higher flies, it has reached this extreme phase by a gradual evolution from a simple condition such as that which prevails in the *Neuroptera*, where all pupal parts are formed inside the corresponding larval parts, as illustrated by Kellogg (19) in studies of *Corydalis cornuta* L.

Among the *Orthorrhapha* the head problem is further complicated by the fact that the larvæ show also various degrees of head retraction. Holmgren (14) and Becker (3) have attempted to explain on this basis the modifications in the *Cyclorrhapha*. But the structure of the parts in this group can not be interpreted as the results of a retraction of the larval head, and the idea has been refuted by all who have studied the embryonic development. Still, while the alteration of the head is described as an "involution," Pratt (41) has shown that what really takes place in the embryo of *Melophagus* is a forward growth of a circular fold of the hypoderm from the rear part of the head (fig. 3, A, x, x) which eventually forms the lips of the new mouth (fig. 3, B, x, x). Processes of growth should probably not be described by such mechanical terms as either

"retraction" or "involution," since they are due to different rates of growth in neighboring parts; but the results in the case of the head of the maggot are equivalent to an involution of the head of the embryo.

The frontal sacs of the mature larva of *Rhagoletis* (Pl. 3, A, E, F, *FS*) are long-stalked pouches extending backward from the ends of the pharyngeal wings (*C*) into the metathorax where they lie against the anterior surfaces of the retracted brain lobes (Pl. 3, F, *Br*). At this stage each sac is distinctly differentiated into two parts, a terminal transverse enlargement containing the bud of the compound eye (*ob*), and a swelling of the stalk which contains the bud of the antenna (*ab*). The buds are thickenings of the walls of the sacs produced into their lumina. Each optic bud receives a nerve from beneath the brain lobe of the same side.

In the embryo of the sheep tick, *Melophagus ovinus*, as described by Pratt (41), a tongue-like lobe filled with vertical muscle fibers projects above the mouth and in front of the origin of the frontal sacs. This lobe is evidently the labrum (fig. 4, A, *Lm*). During the involution of the head it is drawn into the mouth, along with the other parts, and eventually comes to form the roof of the anterior part of the larval pharynx, just posterior to the root of the frontal sacs (fig. 4, B). Its muscles (*Mcl*) now stretch between the roof of the pharynx and the anterior parts of the frontal sacs, and apparently become the pharyngeal dilators of the larva (Pl. 3, A, D, F, *DiM cl*). These muscles consist of two lateral masses (Pl. 3, D) attached above to the inner faces of the wing plates (*C*) of the pharyngeal skeleton, and below to the flexible, invaginated roof of the pharynx (*DPhy*). Their anterior ends lie against the rear wall of the transverse base of the dorsal pouch (Pl. 3, C, *DP*) of the atrium. The contraction of these muscles and the antagonistic flexibility of the pharyngeal roof give the sucking action to the larval pharynx.

A ventral pair of imaginal bud sacs arises from the floor of the atrium in the larva (fig. 3, B; Pl. 3, A, F, *LbB*), which are evidently the buds that are to form the proboscis and the lower parts of the head of the adult. In the embryo of *Melophagus*, according to Pratt (41), these buds arise in the normal external position ventrad to the mouth (fig. 4, A), and are withdrawn into the atrium by the subsequent involution of the larval head (fig. 4, B). Giacomini (12) describes the labial buds of *Eristalis tenax* as arising on each side of the opening of the salivary duct.

It is evident, now, that if the fly were to have mandibles, buds of these appendages should be found somewhere at the inner end of the atrium. But no cyclorrhaphus species possesses mandibles in the adult stage, and no mandible buds have been noted in the larvæ. The oral hooks of the maggot have often been called "mandibles," but if they were true mandibular appendages, they should have been carried into the mouth in advance of the root of the dorsal pouch of the atrium and the bases of the ventral buds.<sup>3</sup> Instead, they arise from a region that is derived either from the original neck or the extreme base of the head. The structure and musculature, too, of the hooks is entirely nonmandibular. They are solid chitinous organs shed with each molt of the larva and are not renewed by the pupa. They arise by wide vertical bases from the cuticle at the inner ends of the lateral pockets of the mouth (Pl. 1, B), where each loosely articulates by a single median knob on its base with the anterior end of the triangular plate (Pl. 3, C, A) of the pharyngeal skeleton. The motion of the hooks is vertical and both work together, though each has its own set of extensor and flexor muscles (Pl. 3, F). Since nearly all the muscles of the maggot degenerate in the pupal stage, the presence of well-developed, special larval muscles for the oral hooks is not evidence that the hooks are derived from primitive

<sup>3</sup> Regarded as parts of maxillæ by de Meijere (Zool. Jahrb., Sysb., Bd. 40, 1915-17, p. 177-322).

structures. They are evidently secondary movable appendages of the cuticle brought forward by the involution process from the back of the head or the neck to the present position at the sides of the new larval mouth.

A recent paper by Bishoff (7) reopens the question of the morphology of the oral hooks in the Cyclorrhapha, but gives no convincing evidence of suggested homologies. Finding some fly larvæ, an unidentified species living in mushrooms, having *four* instead of two mouth hooks, Bischoff presents their case as one of a maggot having both mandibles and maxillæ. He gives no discussion to substantiate such an identification, though he explains at some length that the specimens are not in a molting condition and that neither pair of hooks can, therefore, be explained as those about to be cast off with the slough. Next, he discovers a species having only two oral hooks and a pharyngeal skeleton similar to that of his four-hooked species. Such a combination he finds in *Coenosia*. Then, by comparison, he decides that the single pair of *Coenosia* represent the inner hooks of the other species, and hence the *mandibles*. The weakness of the argument lies in the assumption that any larval mouth hooks are ever either mandibles or maxillæ—the very thing of which we lack the proof. Bischoff makes no reference to the work of Pratt (41) or any other on the actual development of the head and the mouth parts. He is cautious, however, about extending his conclusions to all the Cyclorrhapha, and promises a special paper on the cephalopharyngeal skeleton of this group.

In first-instar Muscoid larvæ there is a small, median, chitinous tooth in the roof of the atrium, which some writers have suggested might be the true mandibles fused into a single rudimentary appendage. The fact, however, that the tooth is situated before the root of the dorsal pouch shows that it, too, belongs to the rear part of the head. The tooth is shed with the first larval molt and does not reappear.

The upper edge of each lateral plate of the pharynx (Pl. 3, C, *B*) and the lower edge of each wing plate (*C*) are deeply incised parallel with their margins. On the lateral plate a cartilagelike ridge (*b*) arises in the marginal notch and turns obliquely downward across the outer surface of the plate. On the wing plate a similar ridge extends from the notch around the posterior edge of the plate, forming a prominent marginal thickening (*c*). These ridges serve for the attachment of muscles (Pl. 3, *F*). The posterior third of each lateral plate and the posterior and dorsal parts of each wing plate are but weakly chitinized and fade off into transparency.

The external musculature of the pharynx is very simple, consisting of four sets of muscles. These are the dorsal protractors (Pl. 3, *F*, *DPMcl*), the lateral protractors (*LPMcl*), the extensors of the oral hooks (*EMcl*), and the flexors of the oral hooks (*FMcl*). The dorsal protractors are inserted on the posterior surfaces of the wing plates (*C*) and along the dorsal edges of their marginal ridges (*c*). Each arises along the mid-dorsal line of the prothoracic segment (*1*) in a row of fingerlike divisions that criss-cross with those of the opposite side. The lateral protractors (*LPMcl*) are inserted along the ventral edge of the marginal ridge (*c*) of each wing plate, and go forward, downward, and outward to the latero-ventral walls of the prothorax, as indicated by the arrow in Plate 4, *C*. The protractors pull the entire pharyngeal structure forward, thus everting the mouth and fully exposing the oral hooks. There are no specific retractors of the pharynx, its retraction being accomplished by the general contraction of the muscles of the body wall (Pl. 4 *C*). The lateral and ventral muscles of the first body segment are inserted anteriorly around the base of the larval head, but six slender dorsal muscles are inserted on the membranous roof of the atrium between the prongs of the triangular plates (Pl. 3, *C*, *a*). The motions of a live



maggot suggest that the anterior parts are extended mostly by blood pressure resulting from the constriction of the rest of the body and the retraction of the rear parts; but when the head and prothorax are fully everted the oral hooks can be still farther protruded by the pharyngeal protractors.

The independent up-and-down motion of the oral hooks is accomplished by their special extensor and flexor muscles (Pl. 3, F, *EMcl*, *FMcl*), which lie against the sides of the lateral plates of the pharynx (*B*), beneath the lateral pharyngeal protractors (*LPMcl*). The two on each side arise posteriorly from the cartilage-like ridge (*b*) of the lateral plate (*B*), and are inserted by narrow tendonlike ends on the upper and lower lobes, respectively, of the wide bases of the hooks.

At the end of the third larval instar, when the hardened puparial skin is separating from the body of the insect within, the hypoderm and new cuticle about the pharyngeal skeleton are loosened and form a sheath over all the external parts of the pharynx and upon the inner faces of the wing plates and the upper surface of the pharyngeal roof. The muscles are thus detached from their skeletal supports. The dilators are retracted from the rear wall of the base of the dorsal pouch (Pl. 3, C, *DP*), being now contained in a tongue of the body cavity lying like a plug loosely in the space between the wing plates and the roof of the pharynx. In this way the cuticular parts are prepared for the final molt, though they remain in place until the second stage of the pupa, when the pharyngeal skeleton and the oral hooks are finally cast out along with the lining of the oesophagus.

All the pharyngeal muscles go into histolysis during the early period of metamorphosis. The protractors and the muscles of the oral hooks apparently have no homologues in the adult, but the dilators may be prototypes of those of the fly.

#### THE ALIMENTARY CANAL AND SALIVARY GLANDS

The alimentary canal and its convolutions are shown in Plate 4, A. The narrow oesophagus (*Œ*) proceeds from the rear end of the pharynx backward between the frontal sacs (*FS*) and the brain lobes (Pl. 3, F, *Br*) to the globular proventriculus (*Pvent*) in the first abdominal segment. The ventriculus (*Vent*), immediately following, has four short gastric caeca (*GC*) on its anterior end, and is disposed in many loops and coils from the first to the seventh abdominal segments, forming most of the length of the alimentary canal. Its final loop meets the intestine at about the middle of the body. The latter makes first one or two coils and then proceeds as a straight tube (*Rect*) to the anus along the left side of the other viscera.

The salivary glands are two thick cylindrical tubes lying latero-ventrally in the front part of the body cavity (Pl. 3, F, Pl. 4, A, *SalGl*). Their ducts unite into a median one (Pl. 3, F, *SalD*) which opens on the floor of the mouth in front of the ventral bridge (Pl. 3, C, *e*) of the anterior lateral plate (*A*) of the pharynx and between the imaginal buds of the labium (Pl. 3, F, *LbB*).

The four Malpighian tubules (Pl. 4, A, *Mal*) arise in pairs from two short basal tubes that originate in small ampullæ on the anterior end of the intestine (Pl. 4, B). In the adult the basal ampullæ of the tubules disappear (Pl. 4, B<sub>1</sub>).

In old larvæ the terminal parts of the Malpighian tubules are commonly much enlarged, often to twice their usual diameter, and are so prominent by their size and opaque white color that they show through the body wall of a live maggot as conspicuous long white bodies in the rear half of the abdomen. This condition of the tubules is due to a great mass of transparent crystals contained in the lumina of the swollen parts. The crystals are structureless bodies varying in size from minute spherical grains to large irregularly oval or ovate stones, 40 microns in diameter (fig. 5, A).

The clear parts of the tubules usually contain only a small amount of minutely granular matter, but occasionally parts of them are filled with crystals of the form shown in figure 5, B. The larger of these, which reach a diameter also of about 40 microns, are clearly concretions about a central body. Wherever they occur the epithelium of the tubule is much reduced.

### THE FAT BODY

The fat tissue of the apple maggot occupies much space in the body cavity, and gives the larva its opaque white appearance. Two large masses of fat cells, however, in the anterior part of the body are particularly conspicuous on account of their pale yellow color. These arise above the salivary glands on each side and meet along the middle of the back. The rest of the tissue forms loose, white masses of a delicate texture, lying along the sides of the body, among the loops of the alimentary canal, and on the floor of the abdominal segments.

The fat cells are very small in young larvæ, and at first contain but little fat. During the third larval stage, however, they increase rapidly in size and their cytoplasm becomes filled with large oily globules. By the middle of this stage the fat cells are mostly from 43 to 46 microns in diameter (fig. 6, A, B), and, at the end of it, they have increased to more than twice this size, being now from 100 to 120 microns in diameter, and are so charged with fat that, in surface view (C), they appear to be mere spheres of oil globules with a surface film of granular protoplasm. When the maggot is contracting to form the puparium the fat cells reach their maximum size, 200 microns or more in longest diameter (D), while the cytoplasm is reduced to branching strands between the voluminous oily inclusions. The granular appearance of the cytoplasm has become more pronounced.

It has been asserted by some writers that the great vacuoles in the fat cells at this stage do not contain fat. Berlese (4) has stated that this is true of the fat cells of the blow-fly, and Koehler (20) says the same for those of the honeybee. Pérez (39), however, claims that the vacuoles in the fat cells of the blow-fly always contain fat, and Bishop (8) says the same of the fat cells of the honeybee.

The present writer found that tests with Soudan

FIG. 5.—A, crystals from the terminal enlarged parts of the larval Malpighian tubules; B, crystals occasionally found in other parts of the tubules, the largest 40 microns in diameter

III and osmic acid on the fat cells of both the honeybee and the apple maggot give very positive reactions in favor of fat in the vacuoles at all stages.

Much of the fat in the fat body of insects with complete metamorphosis disappears during the early part of the pupal period. But it is now known that the fat body of such insects is an organ of much greater physiological activity and importance than its name would imply. Though its cells form mostly fat during the larval stage, which crowds their cytoplasm with oily vacuoles, they store up also glycogen, in some species, and they elaborate proteid or albuminoid bodies which finally accumulate in large numbers in the cytoplasm. For this reason Berlese (4) has appropriately called the fat cells *trophocytes*.

The presence of glycogen in insects has not been generally noted, but Bataillon and Couvreur (2) have shown for the silkworm, and Straus (44) for the honeybee, that the larva stores up glycogen in increasing amount till the end of its life, and that this glycogen is then rapidly consumed during the pupal stage. These investigators, however, did not attempt to locate the glycogen in the body of the

insect; but the fat cells of a mature honeybee larva stain rapidly in iodine and retain a rich, red-brown color after long washing in alcohol. This is the common test for glycogen. The same test gives weaker results on the fat body of a tent caterpillar (*Malacosoma*), and no reaction at all on that of the apple maggot. No one, however, has yet shown by chemical analysis of any fly larva whether it contains glycogen or not.

The accumulation of proteid or albuminoid bodies in the fat cells was described by Berlese (4) for the blow-fly, and has now been noted by all subsequent observers, though the usual test has been that the bodies in question are colored by eosin and other protoplasmic stains. The writer, however, applied the test with Millon's fluid to the fat cells of the honeybee pupa and obtained the proteid reaction with characteristic pink color in the non-fatty inclusions of the cytoplasm.

The fat body, then, is a very important organ in insects with complete metamorphosis, its functions being necessary to the proper reconstruction of the imago, and we can readily understand why an insufficiency of food or an improper diet during the larval period may result in a delay of the time of pupation, in the production of undersized adults, or in the death of the insect during its attempt at transformation.

Some writers have noted, moreover, that, while metamorphosis is in progress, some of the fat cells appear to act as substitutes for the Malpighian tubules when the latter are undergoing reconstruction, for at this period, according to Pérez (39), some of them frequently contain crystals that later disappear. In the honeybee and other Hymenoptera, some of the cells of the fat body are apparently specialized as excretory organs, since they regularly contain small crystalline bodies in their cytoplasm. No such crystalline deposits were noted in any of the fat cells of *Rhagoletis* at any stage.

Other cells, known as *ænocytes*, are usually found associated with the fat cells in insects, and are often regarded also as excretory in function. But the writer did not find such cells in the apple maggot, though they might show in histo-

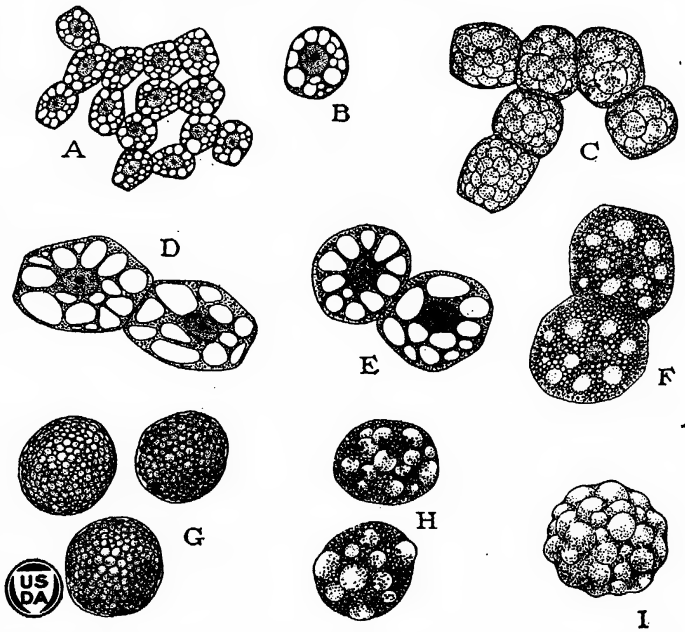


FIG. 6.—Fat cells of the larva and pupa. A, fat cells of half-grown larva of third instar, seen in optical section, the cytoplasm filled with oil droplets; 43–64 microns in diameter. B, a single cell of the same, more enlarged. C, surface view of fat cells of mature larva, showing oil globules crowded beneath surface film of granular protoplasm; 100–120 microns in diameter. D, optical section of fat cells from larva contracting to puparium; 215 microns in longest diameter. E, optical section of fat cells just after formation of puparium, the cytoplasm now coarsely granular with proteid contents, oil less abundant. F, optical section of fat cells 48 hours after formation of puparium, the oil contents greatly reduced, the proteid granules increased in size and numbers; 135 microns in diameter. G, free spherical fat cells of fifth day (115 hours) filled with large proteid grains; 130–150 microns in diameter. H, surface view of fat cells at end of 14 days, proteid contents decreasing, fat increasing in globules that swell out at the surface; 150 microns in diameter. I, surface view of fat cell at end of 18 days, cell full of fat globules bulging out on all sides, in small amount of finely granular cytoplasm; 172–215 microns in diameter.

logical sections. Giacomini (12) describes those of the larva of *Eristalis tenax* as conspicuous cells, arranged in groups along the sides of the abdomen, near the rudiments of the stigmata.

With the beginning of metamorphosis the fat cells begin to decrease in size. When the apple maggot has attained the puparial condition, the oil globules are less voluminous and the cytoplasmic strands are proportionately thicker (fig. 6, E). Evidently the accumulation of fat reserves has passed its peak and consumption has begun. It is to be noted, however, that the granulations in the cytoplasm have become more conspicuous and have clearly grown to be small grains suspended in the plasma.

The further history of the fat cells takes us into the period of metamorphosis. During the first 48 hours after the formation of the puparium the amount of oil in the fat cells continues to diminish, while the granular inclusions of the cytoplasm increase in size till they fill the body of the cell from nucleus to periphery, though, in general, they are largest and most crowded toward the center (fig. 6, F). These inclusions are the albuminoid grains above described. Berlese (4) thought that the albuminoid bodies are formed by cell enzymes given off from the nucleus acting on materials absorbed from the blood, which is now highly charged with the detritus of larval organs. Bishop (8) claims that, in the honeybee, the granules themselves originate in the nucleus, having "presumably the nature of nucleoli"; that, at the beginning of pupation, the nuclear membrane disappears and the nuclear granules pass into the cytoplasm where, by "progressive absorption, both of the surrounding cytoplasmic matrix and of its vacuoles, these granules finally develop into globules containing albuminoid material." Shortly after the beginning of metamorphosis in the apple maggot the fat body breaks up and its cells float off free in the blood and become spherical (G). At the end of the fifth day the cells are mostly 130 to 150 microns in diameter. The albuminoid bodies have now reached their maximum development.

The end of the fifth day after the formation of the puparium marks the time allotted to the pupa for attaining its final external form, though the internal processes of reorganization go on for several weeks. The fat cells themselves enter a period of degeneration. Their shapes become less regular, their proteid grains decrease in size and in numbers, while fat globules reappear in the cytoplasm. But the globules now bulge at the surface as if the cell wall had become too delicate to retain them (fig. 6, H). By the eighteenth day the fat cells are again reduced to masses of oily spheres (fig. 6, I), the droplets swelling out so tensely everywhere that the cell walls rupture under the slightest pressure and allow the cell contents to be scattered in the blood. Remnants of the albuminoid bodies still persist, but they form only a sprinkling of small granules in the scanty cytoplasm.

At last the fat cells break up in the blood by what appears to be a natural dissolution of their membranes. Their contents are disseminated, and the blood plasma becomes filled with oily droplets and masses of the proteid grains loosely held in protoplasmic fragments. At least, the fresh blood of specimens handled in the most careful manner is always highly charged with these elements of broken fat cells.

Nearly all investigators have noted this apparent disintegration of the fat cells during the later part of the pupal period, but some insist that it is due to the preparation or handling of the specimens studied that the fat cells normally remain intact and gradually lose their contents by osmosis through their membranes. Some claim also that most of the fat cells finally fall prey to phagocytes, and that the remainder persist to regenerate the fat tissue of the imago. Scarcely any two writers agree in all details as to the fate of the larval fat cells or the origin of the imaginal cells. The reader may find the various interpretations of observed phenomena in the works of Kowalevsky (24), Van Rees (47), Koschev-

nikov (23), Berlese (4, 5), Terre (45), Anglas (1), and Pérez (38, 39). The writer, in studying the metamorphosis of the fat body of the honeybee, found no evidence of phagocytosis, but was convinced that most of the fat cells do disintegrate, as described by Bishop (8), after a normal dissolution of their membranes. He finds also no suggestion of phagocytosis of the fat body in the apple maggot, but in all cases a disintegration of large numbers of the cells, and this in the blood of fresh specimens removed with utmost care from the puparial shell.

The origin of the fat body of the imago is difficult to determine in Diptera and Hymenoptera, but it appears most probable that it is formed from persisting larval cells, as Bishop (8) concludes in his study of the honeybee. Kreuscher (26) says that in *Dytiscus* the greater part of the larval fat body appears to go over without change into the adult, for the newly emerged beetle often has a fat body with cells rich in fat and full of albuminoid granules, in large part resembling the fat body of the mature larva.

However, by whatever means the larval fat cells finally give up their contents, and whatever may be their ultimate fate, it is evident that they play very important rôles in the metamorphosis of the insect, first, in the capacity of preparers of foodstuffs in the larva and of carriers of the same from larva to pupa, and, second, in that of elaborators of these reserves into food materials for the growing tissues of the adult. In the first case they use the pabulum absorbed from the alimentary canal of the larva; in the second they use the fatty reserves stored in them, perhaps also taking other materials from the blood, formed from the histolysis of the larval organs, and possibly also consuming some of their own protoplasm.

## THE MUSCLES OF THE BODY WALL

Since the maggot has no legs to direct its motions, it must accomplish all its actions by movements of its body, but, so well developed to this end are the muscles of its body wall, that no act of shortening or lengthening, no contortionistic twist or turn, is impossible to it.

Plate 4, C, shows the inner walls of the first four body segments of the maggot cut open from above and spread out flat, with the pharynx turned forward, exposing its ventral surface. Plate 4, E, shows the right half and all of the back of two succeeding segments in the same position but somewhat more enlarged. It will be seen that all the muscles of the body wall lie flat against the skin and that most of them form an intricate network of fibers crossing in all directions. Two latero-ventral bands (*VMcl*) are the only longitudinal muscles of the body. In the first three segments these bands are augmented by extra muscles on each side, which become oblique in the fourth segment.

In general, all the longitudinal and oblique muscles are attached at each inter-segmental line. In the thorax, however, the longitudinals are continuous from the edge of the first abdominal segment (Pl. 4, C, *I*) to their insertions on the rear margins of the larval head. These muscles are thus made particularly effective as retractors of the head and pharynx. In addition, all of one set of oblique muscles of the second segment are continued through the first to be inserted in a circle around the base of the head and on the roof of the atrium. By the combined action of all of these muscles the head can be entirely withdrawn into the prothorax and the latter itself greatly retracted (Pl. 4, D). The lengthening of the body is accomplished by the constrictive action of the oblique muscles and the resulting pressure of the blood and viscera. The oblique muscles cross one another in two regular systems as shown in Plate 4, C and E, forming a pattern repeated in each segment except the first, where one system is omitted. No muscles, however, cross the back, where the heart lies along the midline (Pl. 4, E, *Ht*) in an open space between the muscles on opposite sides.

Another system of weak, superficial muscles lies close to the skin and consists of a few fibers in each segment disposed more transversely as shown in Plate 4, C and E. Finally, along the sides of the body, there is a row of slender transverse muscles (Pl. 4, E, *TMcl*) placed on the intersegmental lines entad to the attachments of the other muscles attached along these lines.

This complicated muscular sheath of the maggot is a special feature of the larva, since few of its fibers are retained in the adult. Most of the larval muscles are either destroyed by histolysis in the pupa stage or form merely a reconstructive basis for muscles of the imago. Van Rees (47) describes only three pairs of muscles in the mesothorax of the larva of the blow fly (*Calliphora*) as persisting into the adult stage, and Breed (10) agrees with him in this, but Pérez (39) describes muscles of the abdomen also as going over into adult muscles, though with complete reorganization. Most investigators, however, agree that in the higher Diptera the majority of the muscles of the adult are built up anew from mesoderm cells of the imaginal buds. Whatever may be the truth with regard to details, however, the musculature of the maggot is a striking example of the degree to which organs may be developed for use during one stage of an insect's life and then discarded or reconstructed to serve the needs of the next stage. The histolysis of larval muscles and the histogenesis of the imaginal muscles will be discussed in the section on metamorphosis.

### THE CIRCULATORY SYSTEM

The heart and aorta are of ordinary tubular form, and are suspended along the midline of the back between the dorsal oblique muscles (Pl. 4, E, *Ht*) in a delicate diaphragm that lies close to the body wall. The writer has not made a detailed study of the circulatory organs of the apple maggot, since they apparently do not differ from those of other cyclorrhaphus Diptera, and must be studied by means of sections. The dorsal vessel of the fly maggot as described by other writers, Pantel (37), Wandolleck (52), Lowne (28), and Giacomini (12), consists of four parts, a posterior section or ventriculus, a median part, the aorta, and an anterior extension of the dorsal wall of the aorta forming an inverted trough-like tongue. The posterior and median sections are contractile and are suspended in the diaphragm, which consists of muscle fibers and the pericardial cells, the latter being larger along the posterior section of the tube than along the median section. Giacomini describes the muscles as ending mesally in branching fibers enclosing the pericardial cells and inserted on the lower lateral parts of the heart.

The most characteristic feature of the circulatory organs of the maggot is the presence of a suspensory ring at the anterior end of the aorta in the neighborhood of the frontal sacs and brain lobes. This was first described by Weismann (53) in the blow fly, and has been the subject of many complicated descriptions. Giacomini (12) says that in *Eristalis tenax* the ring, or suspensory anellus, is composed of epithelial-like cells and slants from above downward and posteriorly. At each end there is a core of small, compressed cells. The ring is anchored at its lower end by two pairs of tendinous cords. One pair goes back to the dorsal wall of the proventriculus, and the other goes forward to the anterior tracheal commissure and neighboring tracheal branches. The aorta, according to Giacomini, penetrates the ring but its lower lip ends immediately in front of it, while its upper half is continued forward past the upper end of the ring to the anterior dorsal commissure of the tracheal trunk, where it ends in membranous ligaments attached to the brain lobes, to the tracheal trunks, to the frontal sacs, and to the pharyngeal muscles. The posterior end of the heart is suspended from the dorsal wall of the body by delicate tendinous fibers.

## THE NERVOUS SYSTEM

The nervous system of the maggot is highly specialized by the condensation of the ventral ganglia. The brain consists of two lobes (Pl. 3, F, *Br*) embracing the oesophagus and connected by a thin bridge above it. There is but one ventral nerve mass, a long, compound ganglion (*Gng*) which is suspended in the body cavity, and extends from the brain to beneath the anterior end of the stomach. This ganglion shows no superficial evidence of its composite origin, except in the numerous paired nerve trunks that arise from it. Only the larger ones of these are shown in the figure.

The unusual position of the brain, in the back part of the thorax (Pl. 3, F, *Br*), is evidently due to the invagination of the primitive head. Since it lies immediately behind the frontal sacs (*FS*) and gives off a short nerve to each optic bud (*ob*), its position is normal in relation to these parts. In the embryo, as shown by Pratt (41), the brain lies in the head behind the frontal involutions. The suspension of the ventral ganglion (*Gng*) appears to be for the purpose of allowing its free motion back and forth with the extension and retraction of the pharynx and the forward parts of the body wall.

## METAMORPHOSIS

The apple maggot attains its full growth in the fruit where it reaches larval maturity by the end of summer. By this time, however, the decay of the pulp, spreading from the maggot's excavations, has caused the apple in most cases to drop to the ground. The mature maggot tunnels outward in the fruit, makes a small exit hole in the skin, emerges, and enters the earth to a depth of from 1 to 3 inches.

Scarcely, however, has the maggot given itself proper burial when its skin begins to turn yellow, to shrink, and to harden. These changes progress till the creature loses all power of movement and has become transformed, by the end of 12 hours, into a motionless, hard-shelled, seedlike object, brown in color, and only 4 or 5 millimeters long, or about two-thirds the length of the active maggot. The insect in this state is usually called a *puparium* (Pl. 5, A, B), but the term should be used to designate only the hardened larval skin which now forms a tough capsule within which the insect will complete its larval life, go through its pupal stage, and transform to the fly. The puparium is, therefore, not a "stage" of the insect's life history. The short length of the puparium as compared with that of the active maggot is due not only to the shrinking of the cuticle, but also to a final involution of the anterior parts, which results in the complete disappearance of the larval head, and in the infolding of nearly all of the prothoracic segment. As a consequence the anterior larval spiracles (*ASp*) come to project from the anterior lateral angles of the puparium.

The external surface of the puparium retains the larval characters, though some of them in modified forms. The segmentation is still evident and the inter-segmental lines are marked by the bands of hooklets, but the swellings have disappeared and the contour is unbroken. Both pairs of larval spiracles are present (Pl. 5, A, *ASp*, *PSp*), but the papillæ about the area of the posterior spiracles are much reduced in size, the smaller ones being scarcely perceptible. The larval mouth is now closed by the hardening of the indrawn parts which form a chitinous plug (*Mth*) in front of the atrium. The anal lobes (*An*) have become densely chitinized and closely appressed, but not fused.

On the other hand certain new characters have developed. During the transformation a ridge swells out on each side of the anterior segments on which there appears a seamlike line (*o*) continuous from one side to the other around the front of the prothorax just below the anterior spiracles. This line ends



posteriorly on each side near the middle of the fourth segment, where it meets a second transverse line (*p*) encircling this segment. These seams are the lines of cleavage along which the puparial shell will be broken open by the escaping fly. Finally, as soon as the puparial cuticle is well chitinized, there appears along each side of the body a row of nine minute stigmata, the primitive lateral spiracles (*Sp*) which have remained rudimentary and invisible during the preceding larval period. The first is located on the methathorax just below the lateral ridge, the next is in the lower angle of the two cleavage lines that meet on the side of the first abdominal segment, and the others follow in line to the eleventh, or eighth abdominal, segment where the last of the series is situated just below the upper of the two lateral papillæ at the sides of the area of the posterior spiracles. These stigmata represent all the adult spiracles except the first, which, though it appears later on the pupa, is apparently obscured by the involution of the prothoracic parts of the puparium.

Though the transformation to the puparium normally goes on beneath the ground, it will take place whether the larva is buried or not so long as it is in an atmosphere sufficiently moist. Hence, for convenience of study, maggots may be collected in a dish as they leave the apples, killed in hot water at desired intervals, and transferred to 85 per cent alcohol, where the puparial shell should be broken. The soft-bodied insect within the puparium can usually be quite easily removed intact, when it is 24 hours old or over, by gently scratching the shell crosswise with a sharp needle till it splits. One end of the capsule can then be taken off and the insect removed from the other.

During the first few hours after the completion of the puparium the hardened cuticle retains its connection with the hypoderm; but at the end of 4 hours in some specimens, later in others, the puparium becomes partly loosened from the inner skin, and after 12 hours it usually forms a free capsule that can be easily removed from the body of the insect within, though it still adheres to the latter at the mouth, anus, and spiracles.

Now, from reading most descriptions of fly metamorphoses, we should expect to find a pupa within the puparium. But the object disclosed in the puparial capsule of the apple maggot at this stage has no character suggesting a pupa. There are no signs of legs or wings; the form is a mere replica of that of the puparium, though smaller in size; and the lateral spiracles are still closed. The new creature, moreover, has a cuticle distinctly its own, characterized, not by any of the former larval characters, but by a uniform, minute papillation all over its surface (Pl. 5, C). Alcoholic specimens show a whitish film between the new cuticle and the loosened puparium, suggesting a coagulated molting fluid. In fact, it is evident that the maggot of the third instar has changed by a puparial molt (*pm*), not to a pupa, but to a *fourth*-instar larva—an intrapuparial, prepupal larval stage (*Ppu*) complete in all respects except for the retention of the stomodeal, proctodeal, and tracheal linings of the third larval instar.

This intrapuparial larval stage of the fly has received scant notice from other writers, and its existence is not generally recognized. Lubbock (29), however, in describing the development of Lonchoptera 60 years ago, said: "When the larva is full grown it detaches itself from the skin, which retains its form, and within which the insect changes into a white, opaque grub, consisting apparently of 13 segments, which gradually diminish in size from one end to the other. There are no limb cases. The skin is covered by small papillæ." But De Meijere (31) would discredit Lubbock's interpretation of what he saw, and suggests that the "grub" inside the puparial skin was the larva of a parasite. A reinvestigation may prove that Lubbock was correct.



The larva of *Rhagoletis* remains in this post-pupal instar a varying length of time, but usually not beyond the end of 48 hours from the time it entered the ground. Then begins another molt which ends in the complete shedding of the papillated cuticle and in the disclosure of the true pupa. But the cast-off integument is not broken; it remains as a delicate, transparent pellicle about the pupa in all its phases of transformation. When the adult fly escapes from the puparium it leaves behind, within the puparial capsule, *two* ruptured skins, the cuticle of the prepupal larva and the cuticle of the pupa, the first characterized by its close-set papillation, the second by a fine surface striation.

The membranous pupal envelope has been noted by other investigators. Künkel d'Herculais (27) says of the Syrphidae: "The pupa is really enclosed in a very delicate skin, a true chitinous cuticle secreted by the hypoderm at the moment of metamorphosis to ensheath all the members. Within this envelope, so transparent that the development can easily be followed through it, the insect organizes itself into the adult."

The shedding of the prepupal cuticle begins at the anterior end of the body (Pl. 5, D, E) and, as the leg buds and the wing buds are everted, the thorax shrinks, thus creating a considerable space inside the loosened membrane. From its first appearance the pupa suggests the structure of the fly with its large mesonotum (Pl. 5, D, *N<sub>2</sub>*) bordered in front by a narrow pronotum and behind by a rudimentary metanotum. The appendages, however, are small at this early period and there is yet no sign of the future head. By the shrinkage of the thorax in the transformation, the tracheal linings (Pl. 5, E, *tra*) attached to the anterior larval spiracles are partially drawn out of the body of the pupa. The new pupal spiracles (*PuSp*) appear between them on the anterior rim of the pronotum (Pl. 5, D, *N<sub>2</sub>*). The pharyngeal skeleton of the larva, however, remains normally still within the pupa, but since it retains its connection with the puparium, it is always extracted with the latter when the puparial shell is removed, leaving a rupture in the prepupa cuticle. For this reason the atrium (*Atr*) appears in Plate 5, E, as an empty, wide-open hole in the front of the thorax.

The molt progresses backward over the abdomen till finally, at the end of the third day, it forms a complete bag (Pl. 5, F, *ppu*) containing the pupa (*Pu*), but still anchored to the latter by the linings of the tracheae, of the stomodeum, and of the proctodeum, and attached to the puparium (*pm*) at the spiracles, mouth, and anus. The abdomen has the prepupal form, but there are no posterior spiracles replacing those of the larva as on the prothorax. There is still no head visible. This phase of the pupal development is the one described by Wahl (51) as the *cryptocephalic* stage.

The pupa remains in this substage (Pl. 5, F) for a variable period of time, and at first can be easily removed from the puparium in the headless form. But, a little later, the slightest pressure consequent on handling causes the head to pop out of the hole in the anterior end of the thorax, when the pupa suddenly assumes the grotesque aspect shown in Plate 6, A. The head is so easily forced out at this period that it can not be demonstrated that its eversion at this time is a normal phase of development, but the appearance of the head marks the beginning of the second substage of the pupa, the one called by Wahl (51) the *phanerocephalic* stage, which in its final phase is shown in Plate 6, C and D.

When the head is first exposed it is very small (Pl. 6, A) as compared with its eventual size (Pl. 6, D). It is a tense, thin-walled capsule with the ptilinum everted in the form of a caplike lobe (*Pt*) on the anterior dorsal aspect. Just below the ptilinum is a transverse, bilobed ridge (*Ant*) within which are developed the antennæ of the fly. On the sides of the head are the compound eyes (*E*), very small at this period, and below each of them a swelling (*u*) which is more developed in the next phase (Pl. 6 C, D), but which is lost in the adult. The

legs and wings (Pl. 6, A) are longer than in the cryptocephalic period (Pl. 5, F), and the thorax shows more structural details of the adult. The abdomen is still very large and completely fills the posterior two-thirds of the puparial capsule.

The walls of the head have been derived from all the parts that were formerly invaginated in the embryo and larva, including the frontal sacs, the atrium, and the dorsal pouch of the latter. The eversion of the head pushes out the skeleton of the larval pharynx, and the entire lining of the pharynx and oesophagus comes out with it; but the larval linings of the tracheae and the intestine still remain within the body of the pupa. When the puparium is artificially removed, however, the intestinal lining usually comes out attached to it, though the dorsal tracheal tubes (*tra*) frequently break loose and remain only partly extracted.

This small-headed phase of the pupa is but a transitory period and is rapidly passed over. In fact, the writer never found intervening phases between it and the next when the head has acquired its final external form and size (Pl. 6, C, D). This arouses suspicion that the evagination of the head at this time may be in all cases a result of the handling of the specimen. Yet the fully formed head is so large in proportion to the size of the thorax that it does not seem as if it could have developed to this degree without a natural eversion at an earlier period of growth.

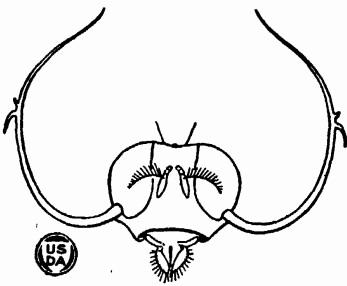


FIG. 7.—Head of an adult fly with subocular horns, *Elaphromyia cervicornis* Saunders. (From Saunders, 43.)

In the final phase of the phanerocephalic pupa (Pl. 6, C, D) the head constitutes almost a third of the entire bulk of the insect. The abdomen has contracted much in size, but still shows the 8 segments of the larva and, in addition, a small anal segment differentiated at the end. The thorax, wings, and legs have greatly increased and lengthened. The ptilinum (*Pt*) is now relatively small, but the bilobed, antennal ridge (*Ant*), the compound eyes (*E*), and the subocular lobes (*u*) are much larger. Each of these last consists of a thick basal part and a tapering terminal spur directed backward. Künckel d'Herculais (27) noted similar subocular processes in the Syrphidae, which he says

are peculiar to the pupal stage in this family, though in several tropical species of *Terastiomys* and *Elaphromyia* they are present in the adult and developed into long appendages. One such species, *T. lobifrons* Bigot, is described and figured by Bigot (6) as having long, bent chitinous processes extending downward from the cheeks. Other forms are described by Saunders (43). One of these, *E. cervicornis* Saunders (fig. 7), has long forked horns like the antlers of a deer; another, *E. alpicornis* Saunders, has large platelike subocular appendages suggesting the horns of a moose. According to Dr. J. M. Aldrich those genera probably belong to the family Micropezidae.

All pupæ of the apple maggot were found to be in the final phase of the phanerocephalic substage at the end of five days, though some mature earlier. Specimens killed at the end of three and four days were found in all of the last three phases of development, the cryptocephalic (Pl. 5, F), the microcephalic (Pl. 6, A), and the macrocephalic (Pl. 6, C). Some of them transformed to flies within a few weeks, while others did not, but this variation is normal, and none was found to remain in a larval stage through the winter, as one writer has claimed.

At some time between the first appearance of the larval head and the attainment of the final pupal form, the larval linings of the tracheæ and usually the lining of the intestine are at last cast out of the body of the pupa. From now on the prepupal cuticle (Pl. 6, G, *ppu*) always shows the molted tracheal linings stuck flat against its inner surface. Those from the anterior spiracles (*ASp*) extend backward as two conspicuous tubes lying just below the level of the

horizontal cleavage lines of the puparium; those from the nine lateral spiracles (*Sp*) form a tangled mass of threads along each side; while those from the hind spiracles (*PSp*) are disposed against the dorsal wall of the envelope in the form of a double-stalked T, as shown in Plate 6, E (*tra*). The extraction of the anterior and the lateral tubes may be accounted for by the contraction of the pupa, but it is difficult to understand how the posterior tubes can acquire their curious arrangement (Pl. 6, E) unless the pupa sways its abdomen sidewise to extract them.

The pupa, now entirely free from its envelopes, retains no connection whatever with any of the larval spiracles, and its own spiracles (Pl. 6, D, *PuSp*) have no direct access to the outside air. In the Anthomyiidae the hornlike pupal spiracles are said to penetrate the puparium between the first and second abdominal segments. In *Rhagoletis*, however, as the body of the pupa contracts during its transformation, the space about it, within the prepupal skin, becomes filled with gas which is visible as a large bubble through the walls of the puparium; and which gives the insect at this stage such buoyancy that it will float on the surface of water. This gas is very evidently the respiratory medium of the pupa, though it is puzzling at first to account for the presence of air in the pupal chamber. An examination of the molted anterior tracheal tubes, however, will suggest the mode of its ingress. These tubes (Pl. 6, F, *tra*), always keep a firm hold on the stigmatic chambers of the spiracles (*ASp*), and the walls of their anterior parts are so rigid that they remain as open cylinders filled with air as far back as the rear margin of the second segment. In the third segment, however, the tubes abruptly collapse to flat bands of flabby tissue into which no air penetrates; but, just before this point, there is always a rupture (*w*) on the inner face of each tube, and these holes establish free passageways from the anterior larval spiracles (*ASp*) of the puparium (*pm*) into the pupal chamber within the prepupal envelope (*ppu*), evidently the inlet of the pupa's supply of air. The slowly diminishing size of the pupa during the period of its transformation may be assumed to suck in the air that forms the first bubble, but subsequent renewal must be by diffusion through the spiracles.

The pupal spiracles differ from the corresponding larval prothoracic spiracles in being bilobed (Pl. 2, A). The stigmatic chamber of each is elongate and forks into the two spiracular lobes where it ends in numerous small papillae that press against the cuticle and appear to contain perforations through the latter. Each pupal spiracle is developed mesad of the preceding larval spiracle (Pl. 5, E), and the remains of the larval stigmatic chamber form outer and inner scars (Pl. 2, A, *m*, *n*) with a cuticular strand (*v*) connecting them as in the larval instars.

After the fifth day the pupa undergoes no further external change. Some pupae will transform to adults in the fall of the same year, but the majority remain in the pupal stage till the following year, when the flies begin to emerge about the first of June, while a few, it is said, do not transform till the second year after the end of the larval stage.

The external forms of the pupa show the progress of the change from the larva to the imago; the real activities of reconstruction go on beneath the surface. The remaking of the insect is not accomplished in the manner by which a piece of clay may be remodelled from one form to another. The processes of metamorphosis are physiological and consist of an active metabolism that affects nearly all the tissues, but not all in the same way or to the same degree. Some parts simply awake from a dormant state and pursue their normal course of development by a sudden access of growth; others that were functional in the larva are altered by direct growth resulting from the activity of their own cells. But the changes most characteristic of metamorphosis are those involving coordinated histolyses and histogeneses resulting in the destruction of larval

tissues and their replacement by new ones generated from the imaginal buds. Finally, some organs undergo histolysis alone and are not replaced, while others are newly formed in the pupa by simple histogenesis. These various processes, however, as we shall see, are probably not so diverse as they have seemed.

The best examples of parts that follow the path of normal development from embryo to imago are the organs of reproduction. But even here growth is almost arrested during the larval period. In the pupa the organs resume activity, but in the case of the apple maggot, according to Illingworth (15), the ovaries of the female fly do not produce mature eggs till from 14 to 24 days after emergence.

The heart, the nervous system, and the tracheal system undergo more or less change in the pupa in order to arrive at the forms they are to have in the fly. Yet, in most cases, the metamorphosis of these organs is a matter of direct transformation by the growth of their own tissues. Alterations of this sort take place also in insects with incomplete metamorphosis, and constitute a bond of unity between the young of such insects and the maggot, grub, or caterpillar of insects with metamorphosis erroneously called "complete."

The larval hypoderm, salivary glands, and alimentary canal have their tissues destroyed but replaced in situ by new ones proliferated from the imaginal buds, and such regenerated parts often take on forms strikingly different from those of the larva. Many of the muscles undergo a similar metamorphosis, though the new tissues have never been traced to imaginal muscle buds, being formed by a reorganization of the elements of the old muscles. This process, however, is apparently not confined to the pupal stage, for Mobusz (35) has described the regeneration of the stomach epithelium in the larval instars of a beetle (*Anthrenus*) as well as in the pupa. It may be suspected, therefore, that reconstruction may occur also in insects with incomplete metamorphosis.

Many of the larval muscles, especially in the higher *Diptera*, are completely destroyed by histolysis and are not replaced by corresponding new ones. On the other hand, some muscles that have no larval prototypes are newly generated in the pupa. Those of this type most probably arise from the mesoderm layer of the imaginal discs of the body wall. The destruction and regeneration of muscles must take place also in some insects with incomplete metamorphosis, but the histology of the process has not been studied in this group.

The metamorphosis of the hypoderm and of the alimentary canal has been described by so many writers, who agree on most of the details, that only a general statement need be made here. The writer has not studied the subject in *Rhagoletis*. At the beginning of metamorphosis the ectodermal cells of the imaginal buds of the hypoderm begin to divide and multiply. Wherever there are appendages the latter grow rapidly, but the peripheral parts of the buds spread out and constitute the new hypoderm of the body wall. The old cells and the basement membrane are absorbed into the blood or are devoured by phagocytes as the new tissues crowd upon them and take their places. This is subsequent to the loosening of the larval cuticle, though the latter may not be cast off till some time later.

Likewise, the epithelium of the foreintestine and of the hind intestine is replaced in the same way from imaginal buds in the intestinal walls. In the mid-intestine, or ventriculus, however, the larval epithelial cells are shed into the lumen, and the imaginal epithelium is regenerated from groups of deep-seated regenerative cells. According to Rengel (42) these regenerative cells in a beetle (*Tenebrio molitor* L.) are the same that also regenerate the ventricular cells lost during the process of digestion in both the larval and the adult periods. The new epithelium proceeds at once to digest and absorb most of the discarded larval epithelium.

All of these tissues are regenerated without destroying the continuity of the organs concerned. The pupa is never without a hypoderm, never without an alimentary canal (Pl. 6, B). The new cells advancing from the imaginal buds follow closely on those disappearing, or overlap them. The new forms of the organs result from the forces of heredity inherent in the new cells or somehow imposed upon them.

In the organs of mesodermal origin, however, especially in the muscles, the tissues are much more profoundly affected by the processes of metamorphosis; yet, even here, in the simpler forms of metamorphosis, the muscles are mostly remodelled *in situ*, and in the higher flies, where the general dissolution is most intense, some of the muscles are said to be modified only by the addition of new fibers. Most of the larval muscles in the Muscoidea, however, go into complete histolysis, while the new ones are built up with little relation to those of the larva.

In the apple maggot the beginning of histolysis in a muscle is first evident in a few fibers that lose their cross striation and appear to melt together into a homogeneous granular mass. This mass then breaks up into small fragments, which are at first densely packed but which are soon dissociated by a clear substance that appears among them as if formed from the liquefying of some other parts of the muscle tissue, probably the sarcoplasm. In this way the muscle disintegrates, resulting in the appearance of clear plas-

mic areas along its edges and between its fibers, filled with granular fragments of the formerly striated tissue. As dissolution progresses deeper and deeper into the body of the muscle, the plasmic lacunæ enlarge and engulf an ever-increasing number of the fragments. Finally, the lacunar plasma dissolves along its outer edges and the muscle fragments float off free into the blood.

Among the bodies in the plasmic areas of a disintegrating muscle we may distinguish several sorts. The most numerous are simple, oval, granular pieces of the fiber substance, the *sarcolytes* of other writers. Then there are smaller bodies containing nuclei, virtually nucleated cells derived from the muscle tissue. These are the *caryolytes* of Berlese (4, 5). Finally, there are often large numbers of free muscle nuclei, or nuclei surrounded by only a very small amount of clear protoplasm. Figure 8, A, shows a piece of a larval pharyngeal muscle in which histolysis is well advanced, and in which is seen the formation of the various elements of disintegration, first deep in the muscle tissue, then liberated into the

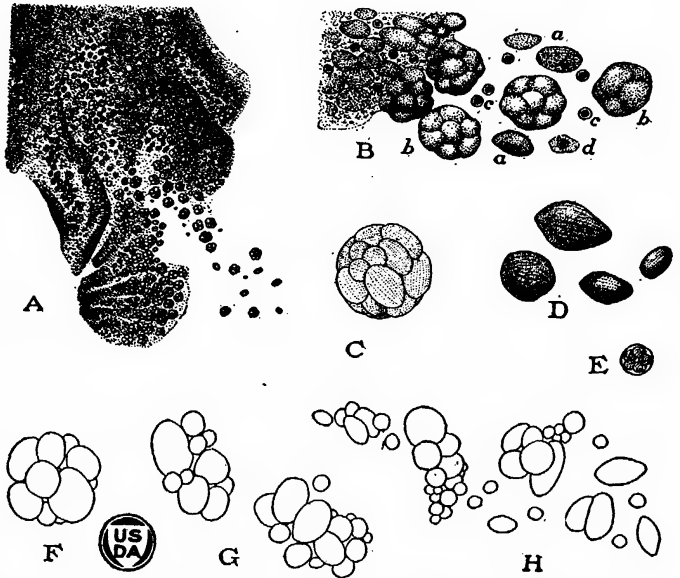


FIG. 8.—Histolysis of muscle tissue. A, piece of pharyngeal muscle in histolysis just after formation of puparium, showing disintegration of muscle into fragments or *sarcolytes* and the liberation of the latter at the dissolving edge. B, showing various elements given off from muscle in histolysis; a, free muscle fragments or sarcolytes; b, globules of fragments; c, free muscle nuclei; d, a muscle "cell" or muscle nucleus in a piece of muscle substance. C, a globule of sarcolytes, about 34 microns in diameter. D, free sarcolytes or muscle fragments, 25 to 30 microns in length. E, cell containing several small bodies, perhaps a free muscle nucleus after division into secondary nuclei, 8 to 10 microns in diameter. F, sarcolyte globule in early stage of disintegration. G, globules in more advanced stages of disintegration. H, globules in last stages of breaking up into free sarcolytes

clear plasma of the lacunæ, and finally given off at the dissolving edge of the latter into the blood.

The different kinds of bodies given off from the edge of a dissolving muscle are shown enlarged at B in figure 8. Here are seen free sarcolytes (*a*), a caryolyte (*d*), and free muscle nuclei (*c*, *c*). It is to be noted, however, that the majority of the sarcolytes are given off in small globular masses (*b*, *b*) which are extruded from the muscle, apparently in a thin matrix of plasma. These bodies soon become spherical (*C*) and are very suggestive of the *Körnchenkugeln* or *spherules of granules* of other writers.

A "Körnchenkugel" is supposed to be a leucocyte or amoeboid blood corpuscle gorged with the fragments of disintegrating larval muscles, which is to say, it is a phagocyte after a full meal. The term was first used in this sense by Weismann (53), but the idea has given rise to an endless discussion in the works of subsequent writers on insect metamorphosis. The present writer will not attempt to give a history of this subject here, since the reader will find in almost every other recent paper a good review of the results and conclusions of all other writers, and, if sufficiently interested, should consult the works of Weismann (53), Ganin (11), Metschnikoff (32), Van Rees (46, 47), Kowalevsky (24, 25), Rengel (42), Karawaiew (17), Needham (36), Anglas (1), Berlese (4, 5), Breed (10), Pérez (39), and other papers by these same and other writers which are listed in all bibliographies on insect metamorphosis.

Some writers have believed that the leucocytes, either acting as phagocytes or by giving off some solvent (*lyocytosis*, Anglas, 1), are the actual cause of the destruction of the larval tissues when the latter reach a condition where their vitality is no longer sufficient to withstand the attacks of these ravenous or virulent cells. It has been shown by Berlese (4, 5), however, that even in the higher Diptera muscles are frequently to be seen in histolysis when the sarcolemma is yet intact and, therefore, before the leucocytes could attack their tissues. Besides this, it is well known that in many insects the metamorphosis of the tissues is not accompanied by phagocytosis. Karawaiew (17) says that phagocytes play a very unimportant part in the metamorphosis of the ant, while Korotneff (21) claims that there is a complete absence of phagocytosis in *Tinea*, and Rengel (42), Needham (36), and Breed (10) report the same for various beetles.

Even in the Diptera the presence of phagocytes is not universal. Miall and Hammond (34) say that they "have never seen in *Chironomus* larval muscles excavated by phagocytes, nor fragments of striped muscles lying inside phagocytes, though both can be demonstrated in the blow fly. In *Chironomus* the disintegration of the larval organs of the thorax is relatively slow, and the muscles, for instance, seem to waste gradually and uniformly while undergoing for a long time no marked change of external form." Kellogg (18) has shown also that in *Holorusia* and in *Blepharocera* histolysis takes place without the occurrence of phagocytosis, and Berlese (5) says the same of *Mycetophila*. Yet Pérez (39) still insists that, in the blow fly, phagocytosis is at least synchronous with histolysis, and he inclines to the belief that the attack of the leucocytes is the actual cause of the destruction of the muscles. Probably most investigators, however, now regard the leucocytes as mere scavengers that devour the products of histolysis, the latter resulting from some other unknown cause. Most writers believe that the phagocytes digest the engulfed particles and give the products back to the blood in a form that can be utilized by the growing tissues. Berlese, on the other hand, claims that they act as carriers only.

Nearly all writers have admitted that, where the leucocytes play the part of phagocytes in the higher Diptera, they form the bodies known as "*Körnchenkugeln*" or "*spherules of granules*;" but Berlese has shown that similar bodies



may be formed from aggregations of sarcolytes, though he claims that such bodies contain at least one nucleated fragment. Janet (16), however, says that in the Muscidae some of the spherules are mere masses of sarcolytes which may or may not contain a muscle nucleus.

In the pupa of *Rhagoletis* there are great numbers of spherules of muscle fragments (fig. 8, C) of the sort described by Janet, and the writer did not certainly find any containing nuclei. Moreover, many of these spherules are formed deep in the body of the disintegrating muscle (A) where no leucocyte could get at them. They originate as mere groups of sarcolytes that adhere in small masses as the sarcoplasm dissolves about them, and that have been thus set free with a thin plasmic matrix cementing their elements together in globular form. Furthermore, leucocytes are always scarce; never are they found in such numbers as to account for the great mass of spherules that is eventually given off into the blood.

The sarcolytes have been described by nearly all writers as retaining the original striation of the muscle fibers. In *Rhagoletis* the muscles lose their striation at the beginning of histolysis, and the sarcolytes are at first granular, though they soon acquire a striated appearance (fig. 8, C, D). Their "striation," however, is clearly secondary and appears to be due to a puckering of the surface, since the lines are always lengthwise and converge at the poles of the oval fragments. The material studied was taken from muscles or from drops of the body liquid of freshly killed larvæ. The specimens were not fixed or hardened, and some were examined without staining, in order to be sure that nothing could be attributed to the effects of reagents.

The bodies derived from the disintegrating muscles soon become very abundant in the blood. The spherules of sarcolytes are the most numerous and the most conspicuous, and most of them remain intact for a long time. After the pupa has acquired its final external form, however, they begin to disintegrate in a manner similar to that by which the fat cells go to pieces. They first lose their even contours (fig. 8, F), and later appear in very irregular forms or as mere loose aggregations of sarcolytes (G). This condition results evidently from a dissolving of the plasmic matrix that so far has held the component fragments together. By the fourteenth day after the formation of the puparium most of the sarcolyte spherules are in these various stages of disintegration.

At about this time the blood of the pupa has a thick, granular, creamy consistency, so charged is it with the débris of both the muscles and the fat cells. Among this material there may be distinguished fat cells still intact, fat cells in various stages of disintegration, free oil droplets, masses of proteid granules from the fat cells, sarcolyte spherules still intact, spherules in all stages of dissolution, free sarcolytes, caryolytes, and free muscle nuclei. In addition, there are leucocytes, various unidentified cellular elements, and great numbers of small grains of indeterminable origin.

At the end of 18 days the sarcolyte spherules have been mostly broken up, and only free sarcolytes or dissolving groups of them (H) remain. The fat cells have likewise in large part disappeared. If the pupa is to become a fly in the fall of the same year, the contents of the blood are now rapidly dissolved; but with those that will not transform till the following summer, the processes of absorption are slower, and by the first of December the blood is still thick with the residue of larval tissues.

Frequently there are to be seen in the blood small cells of the size of muscle nuclei, which contain several smaller bodies (E). These are evidently what Berlese (5) calls *sarcocytes*, or larval muscle nuclei that have divided by direct division into a group of small nuclei. These secondary nuclei, he says, become

liberated and grow into elongate migratory cells, the *myocytes*, which gather at the points where new muscles are to be, or group themselves with the remaining parts of old muscles, to build up the muscular system of the imago. Anglas (1) described the imaginal muscles of the wasp as originating from *myoblasts*, or fragments of the larval muscle nuclei in pieces of the contractile tissue.

Ganin (11) and Van Rees (47), however, had described the imaginal muscles of the blow-fly as formed in part or entirely from cells proliferated from the mesodermic tissue of the imaginal buds of the body wall. Pérez, in a more recent study of the metamorphosis of the blow-fly (39), reasserts this same view concerning the origin of the new muscles or of new parts of old ones, and claims that the larval muscle nuclei finally perish by phagocytosis.

One of the most convincing papers on the metamorphosis of the muscles is that by Breed (10) in which are described the changes that occur in the muscles of certain beetles. In the species studied, Breed found no evidence of phagocytosis, and, according to his observations, muscles that metamorphose are reconstructed from their own elements, not from migratory "myocytes," while muscles of new formation are probably developed from the mesoderm of the imaginal buds. Cells of external origin do penetrate the growing muscles, but these, he points out, are derived from neighboring tracheole cells and form the imaginal tracheal branches of the muscles. Such cells, Breed suggests, are the ones observed by Berlese and described as migrating "myocytes" on the mistaken belief that they formed the new elements of the muscles. Breed's account agrees entirely with that of Rengel (42) on the metamorphosis of the muscles of the alimentary canal. Needham (36) says that some of the nuclei of the degenerating fat cells of the flag weevil become associated with the developing muscles, apparently themselves becoming nuclei of the new muscle fibers. This, however, seems no more reasonable than Berlese's idea, later discarded by himself, that some of the muscle nuclei form the imaginal fat cells.

The muscles of any pupa, then, may be classed in not more than four groups, as given by Breed (10). The first includes such muscles as pass unaltered from larva to imago, but most writers agree that there are no muscles of this sort in the higher Diptera. The second group includes such larval muscles as undergo a more or less complete metamorphosis by a reorganization of their own elements. Van Rees (47) described three pairs of larval muscles in the thorax of the blow-fly that persist with alterations, and this is confirmed by Breed. The third group consists of those larval muscles that disappear by complete histolysis. This includes most of the larval muscles of the Muscoidea. The fourth group consists of muscles that are newly formed in the pupa from pre-existing imaginal buds, and includes most of the muscles of the adult fly.

The muscles of new formation are to be regarded as present in rudimentary form in the imaginal buds. These muscles are those that the larva has found unnecessary or unsuited to its purposes, and, as stated by Breed, "such muscles would tend naturally to be retarded in their development until they came to be muscles newly formed in the pupa; but in their final development they would arise from the cells which had originally formed them." It has not been shown yet just what elements in a metamorphosing muscle generate the new parts, but the evidence is against the idea that regenerating elements enter from without, and Breed sees no evidence that the new elements in such cases are derived from the imaginal discs. Perhaps each muscle, then, contains cells with persistent vitality that regenerate it, just as the hypoderm and the walls of the alimentary canal are regenerated by cells in their own tissues that have remained dormant through the larval stage or that have retained their vitality into the pupal period.

## SUMMARY

By assembling all available information on the comparative anatomy, embryology, and postembryonic development in the Diptera, we may construct the following outline of the evolution of one of the higher flies, and thus arrive at a better understanding of the structure and metamorphosis of a maggot than could be gained from a study of the maggot alone.

1. The larvæ of primitive flies had legs and a normal head with antennæ and biting mouth parts, and breathed through lateral spiracles connected with the lateral tracheal trunks.

2. As a result of the reduction of the external appendages in the larval stage, the corresponding appendages of the pupa were forced to back into the body in order to find space for growth, thus coming to be developed in deep pockets of the hypoderm.

3. As long as there was a remnant of the larval appendage, the tip of the pupal appendage developed within it. When the larval appendage disappeared entirely the bud of the pupal appendage became free in its hypodermal pouch. The bud was then at liberty to begin its growth in earlier larval instars or even in the embryo. Thus has been established the precocious origin of imaginal buds in the Diptera. Yet the imaginal rudiment, regardless of when it begins its growth, is morphologically of the pupal stage—the hypoderm pouches of the buds, the peripodal sacs, shed no cuticle, so far as known, till the molt of the pupa.

4. Imaginal buds of the body wall that do not form appendages, such as most of those of the abdomen, remain as flat discs of cells in the hypoderm, typically a dorsal and a ventral pair in each segment in line with the appendage-forming buds of the thorax.

5. In the higher Diptera the imaginal buds of the antennæ and the compound eyes are developed in a single pouch on each side of the head, the so-called frontal sacs.

6. By an invagination of the frontal region the roots of the frontal sacs were carried inward. These new cavities then united basally, forming a median bilobed pouch with the true frontal sacs arising from the inner ends of its wings.

7. The head of the larva suffered a reduction after the loss of its appendages, and then suffered an involution resulting either from a retraction of the mouth or from a forward growth of a fold from the base of the head. The inturned parts thus came to form an anterior addition to the pharynx, known as the head atrium; and the median frontal pouch, carried in at the same time, formed the dorsal pouch of the atrium. The two wings of the pouch still carry the bases of the original frontal sacs, which last are buried deep in the thorax along with the brain lobes retracted in advance of them.

8. By the involution of the head, the labrum and the buds of the mouth parts were also carried inward. The labrum now forms the roof of the atrium behind the root of the dorsal pouch, while its muscles constitute the dilators of the new larval pharynx. The buds of the mouth appendages arise from the floor of the atrium.

9. The maggot early lost its true mandibles, but later acquired substitutes in the form of hooks located at the sides of the new mouth. Since this region came from the back of the head, or from the neck, the oral hooks appear to be secondary cuticular organs. They move in a vertical plane and are solid structures shed with each larval molt.

10. In connection with the oral hooks there was developed a chitinous skeleton in the walls of the atrium, the dorsal pouch, and the original larval pharynx, thus unifying all these parts in a structure designed to support the hooks and to give attachment to muscles for moving them. This skeleton is also renewed with each larval molt.

11. The original maggots perhaps took to living in soft mud or in water, and, in adaptation to their new environment, they developed a pair of dorsal spiracles at one or both ends of the body in connection with the *dorsal* longitudinal tracheal trunks, while the primitive spiracles of the *lower* or *lateral* tracheal trunks were closed. They thus became propneustic, metapneustic, or amphipneustic by a new breathing system, *not by a modification of the primitive peripneustic system*. The dorsal spiracles are independently renewed with each molt.

12. The puparium of the higher Diptera is the hardened skin of the last active larval instar. In *Rhagoletis* it is loosened from the underlying hypoderm, and a fourth larval instar is formed within the puparial shell. This larva has a minutely papillated cuticle of its own, but it does not shed the linings of the tracheæ and alimentary canal of the third instar. This prepupal larva soon molts in its turn, whereupon the true pupa is formed, and its unbroken cuticle remains as a membranous envelope about the pupa within the puparial capsule. How far this condition is general in the Diptera has not been determined.

13. In the pupal stage all the sunken imaginal parts are everted, including the frontal sacs and the buds of the mouth<sup>ly</sup> parts, legs, and wings. Structures that have been acquired by the larva for its own special purposes are lost by histolysis; those that have been modified are remodeled into imaginal organs; and imaginal organs or tissues that have been suppressed grow rapidly into the adult form. In the higher Diptera the pupa goes through two external phases in its development, a cryptocephalic substage and a phanerocephalic substage.

14. The pupa supplants the anterior dorsal larval spiracles with a corresponding pair of its own, but it loses, without replacing, the posterior larval spiracles. In *Rhagoletis* the pupa obtains air from the anterior larval spiracles of the puparium conducted into the pupal chamber through the cast larval tracheal tubes which remain connected with the anterior larval spiracles and which are ruptured within the prepupal envelope of the pupa.

15. The pupal metamorphosis is for the purpose of restoring the ancestral form of the insect, but it involves a restoration complicated by the addition of all the special characters added by the adult during its own evolution. In general, the more the larva has departed from the ancestral path, the more the adult has modified its inheritance, and consequently the more arduous becomes the work of the pupa in making the one over into the other. Nowhere in insects has this independence between young and adult been carried to such a degree as in the higher Diptera, and consequently a more intense reconstruction goes on in the fly pupa than in any other.

16. Physiologically the life of the insect is divided into but two periods—the larval period and the imaginal period. The pupa is a constructive proimaginal stage separated from the mature imaginal stage by a secondarily acquired molt. It is not a preimaginal phylogenetic stage. In most cases it has developed special characters of its own, and often retains one or more special characters of the larva.

17. The adult represents the primitive form of the insect on which has been built up the special adult characters acquired by each species in its evolution. In insects with metamorphosis the adult restores characters of its ancestors that were discarded by the larva. In the higher Diptera, for example, the fly abolishes the breathing system established by the larva, reduces the enlarged dorsal tracheal trunks to normal size, and reopens the primitive lateral spiracles along the lower lateral tracheal trunks. These spiracles remain through the larval stage as mere groups of special cells in the hypoderm, which appear first, in *Rhagoletis*, as minute stigmatic spots on the puparium. The imago of insects with complete metamorphosis is not a new creature built up independently from embryonic cells; its first stage, the pupa, follows the last larval stage by a process of replacement which is only a modification of that which takes place between any two larval stages, and which again occurs in normal form at the molt between the pupa and the adult.

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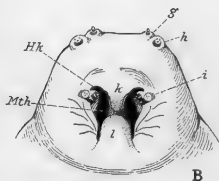
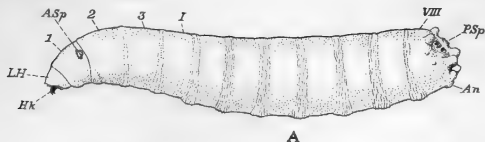
## PLATE 1

### *Rhagoletis pomonella*

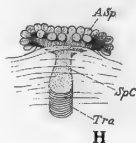
- A.—Lateral view of mature larva in third instar.  $\times 10$ .
- B.—Ventral view of larval head, showing mouth, oral hooks, and sense organs.
- C.—Sense organs of snout of larva, greatly enlarged.
- D.—Vertical view of same sense organs as in C.
- E.—Sense organ at angle of mouth with pair of chitinous teeth on adjacent edge of lip. (See B.)
- F.—Part of an intersegmental band of hooklets on cuticle of larva.
- G.—Two hooklets seen in profile.
- H.—Anterior spiracle of second-instar larva, lateral view.
- I.—Diagrammatic dorsal view of anterior spiracle of third-instar larva, showing closed passage from base of stigmatic chamber to cuticle of body wall.
- J.—Lateral view of anterior spiracle of third-instar larva.
- K.—Three stigmatic papillæ of anterior spiracle of third-instar larva, showing terminal slit in each.
- L.—Oral hook of first-instar larva.
- M.—Oral hook of second-instar larva.
- N.—Oral hook of third-instar larva.

### EXPLANATION OF SYMBOLS

- 1, prothorax.
- 2, mesothorax.
- 3, metathorax.
- I, first abdominal segment.
- VIII, eighth abdominal segment.
- An, anus.
- ASp, anterior larval spiracle.
- BW, body wall.
- g, anterior sense papilla on snout of head.
- h, posterior sense papilla on snout of head.
- Hk, oral hook.
- i, sense organ at side of mouth.
- j, teeth on edge of lateral hook pocket of mouth.
- k, median ridge on roof of larval mouth.
- l, median ridge on floor of larval mouth.
- LH, larval head, external remnant of invaginated true head.
- m, outer stigmatic scar, or closed outer end of passage through which tracheal cuticle of preceding instar was shed with cast-off spiracle.
- Mth, larval mouth.
- n, inner stigmatic scar, in base of stigmatic chamber. (See m.)
- PSp, posterior larval spiracle.
- SpC, stigmatic chamber.
- Tra, dorsal longitudinal tracheal trunk.
- v, remains of passage of stigmatic chamber to body wall, through which tracheal intima of last larval instar was shed. (See m and n.)



F

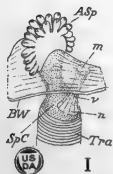


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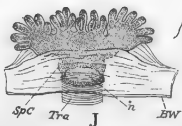
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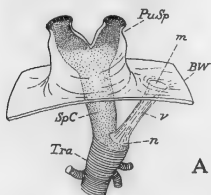
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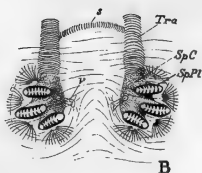
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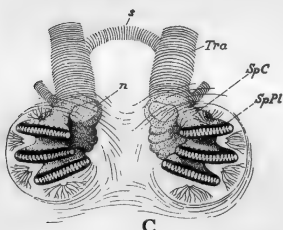
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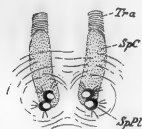
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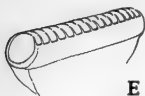
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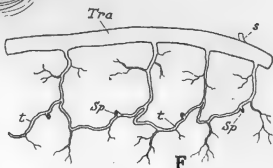
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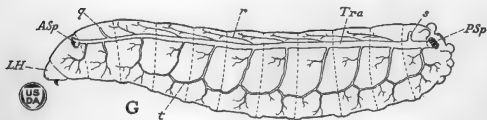
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E



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G

## PLATE 2

*Rhagoletis pomonella*

- A.—Dorsal view of right pronotal spiracle of pupa, showing passageway from base of stigmatic chamber to cuticle through which larval tracheal intima was shed attached to larval spiracle.
- B.—Posterior spiracles of second-instar larva.
- C.—Posterior spiracles of third-instar larva.
- D.—Posterior spiracles of first-instar larva.
- E.—Stigmatic plate at end of one lobe of stigmatic chamber of third-instar larva.
- F.—Dorsal tracheal trunk and lateral branches of last four segments of pre-pupal larva, showing lateral spiracles connected with lateral commissures, which will constitute the lower lateral longitudinal trunk of the adult tracheal system.
- G.—Principal trunks and branches of left half of larval tracheal system, third instar.

## EXPLANATION OF SYMBOLS

- ASp*, anterior larval spiracle.
- LH*, larval head, external remnant of the invaginated true head.
- m*, outer stigmatic scar, or closed outer end of passage through which tracheal cuticle of preceding stage was shed with cast-off spiracle.
- n*, inner stigmatic scar, in base of stigmatic chamber. (See *m*.)
- PSp*, posterior larval spiracle.
- PuSp*, pupal dorsal spiracle of pronotum.
- q*, anterior dorsal tracheal commissure.
- r*, V-shaped dorsal tracheal commissures. (Only left halves shown.)
- s*, posterior dorsal tracheal commissure.
- sp, sp*, lateral spiracles, the adult system, connected with lower longitudinal tracheal trunks.
- SpC*, stigmatic chamber.
- SpPl*, stigmatic plate.
- t, t*, tracheal commissures forming lower lateral longitudinal trunks of adult.
- Tra*, dorsal longitudinal tracheal trunk.
- v*, remains of passage from stigmatic chamber to body wall, through which tracheal intima of last stage was shed. (See *m* and *n*.)

# PLATE 3

## *Rhagoletis pomonella*

A.—Pharyngeal skeleton and associated parts, lateral view, mature larva of third instar.

B.—Larval pharynx and left oral hook. Same as A with soft parts removed.

C.—Larval pharynx and skeleton with oral hooks, diagrammatic.

D.—Transverse section of larval pharynx and pharyngeal skeleton through bases of wing plates, anterior view. (Compare with C.)

E.—Dorsal view of pharyngeal skeleton, with oral hooks and frontal sacs, third-instar larva.

F.—Vertical, lengthwise section of head and first four segments of mature maggot, showing pharyngeal skeleton and musculature, the frontal sacs against the brain lobes, imaginal buds of labium and legs, ventral ganglion, salivary glands, and anterior parts of alimentary canal (*Æ*, *Pvent*, *Vent*).

### EXPLANATION OF SYMBOLS

1, prothorax.

2, mesothorax.

3, metathorax.

I, first abdominal segment.

A, anterior lateral or triangular plate of larval pharyngeal skeleton.

a, arms of plate A in roof of atrium.

ab, antennal bud in frontal sac.

Atr, atrium, anterior, part of larval pharynx resulting from involution of original head of larva.

B, posterior lateral plate of pharyngeal skeleton.

b, ridge on plate B of pharynx.

Br, brain.

C, dorsal or wing plates of pharyngeal skeleton, developed in arms of dorsal pouch of atrium.

c, ridge on plate C of pharyngeal skeleton.

D, bridge plate of pharyngeal skeleton in roof of atrium.

d, vertical, longitudinal folds of floor of pharynx.

DiMcl, dilator muscles of pharynx.

DP, dorsal pouch of atrium, divided beyond base into two wings containing plates C of pharyngeal skeleton and leading to roots of frontal sacs.

DPMcl, dorsal protractor muscles of pharynx.

DPhy, dorsal wall of pharynx.

e, chitinous bridge in floor of atrium connecting pharyngeal plates A.

EMcl, extensor muscles of oral hooks.

f, two small sclerites in floor of pharynx just behind the mouth.

FMcl, flexor muscle of oral hook.

FS, frontal sacs, containing imaginal buds of antennæ and compound eyes.

G, pupal hypoderm separated from larval pharyngeal skeleton. (See A.)

g, anterior sense papilla on snout of larval head.

GC, gastric caecum.

Gng, ventral ganglionic nerve mass.

h, posterior sense papilla of snout of larval head.

Hk, Hks, oral hook, oral hooks.

L<sub>1</sub>, L<sub>2</sub>, L<sub>3</sub>, leg buds of prothoracic, mesothoracic, and metathoracic legs, respectively.

LbB, imaginal buds of labium.

LH, larval head.

LP Mcl, lateral protractor muscles of pharynx.

Mth, larval mouth.

op, optic bud, imaginal bud of compound eye.

Æ, oesophagus.

Phy, lumen of larval pharynx.

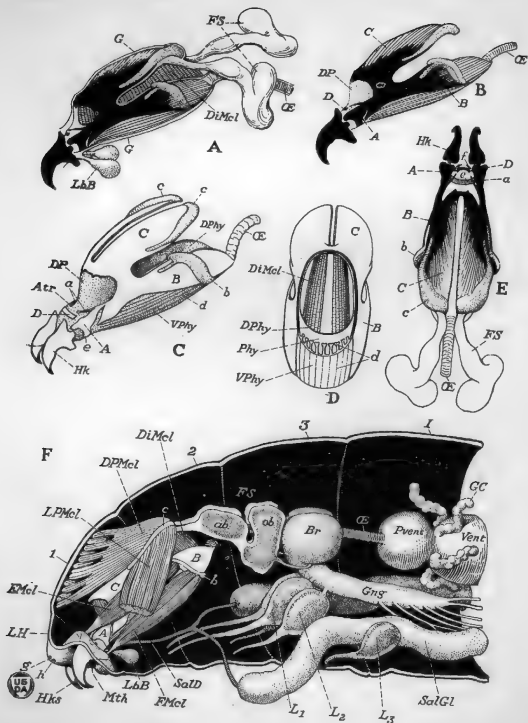
Pvent, proventriculus.

SalD, salivary duct.

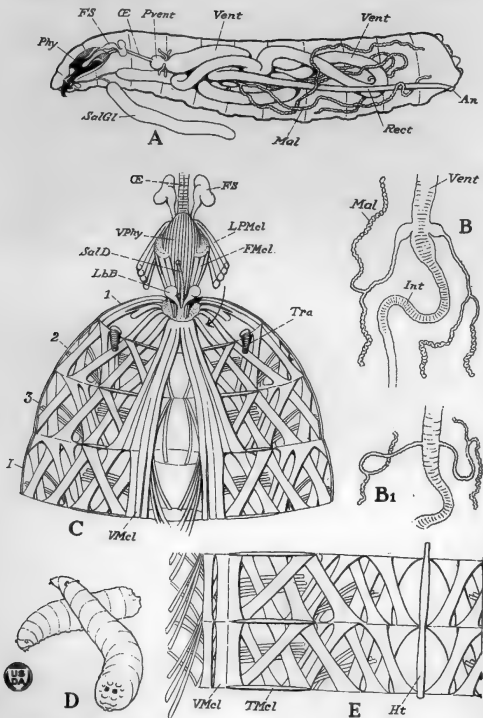
SalGl, salivary gland.

Vent, ventriculus.

VPhy, ventral wall of pharynx.







## PLATE 4

### *Rhagoletis pomonella*

A.—Outline of mature maggot, showing position of larval pharynx, alimentary canal, and salivary glands.

B.—Union of stomach and intestine of larva, showing bases of Malpighian tubules.

B<sub>1</sub>.—The same parts in the adult.

C.—Musculature of ventral and lateral parts of first four segments of mature larva, showing pharynx turned forward and the lateral pharyngeal protractor muscles separated from attachments on body wall as indicated by arrow on right. The roots of salivary duct and labial buds seen below anterior end of pharynx.

D.—Maggots in attitudes of extension and retraction of anterior segments.

E.—Musculature on right side and back of two consecutive segments, more enlarged than in C, but in corresponding position.

### EXPLANATION OF SYMBOLS

1, prothorax.

2, mesothorax.

3, metathorax.

I, first abdominal segment.

An, anus.

FMcl, flexor muscle of oral hook.

FS, frontal sacs, containing imaginal buds of antennæ and compound eyes.

Ht, heart.

Int, intestine.

LbB, imaginal buds of labium.

LPMcl, lateral protractor muscles of pharynx.

Mal, Malpighian tubules.

Æ, œsophagus.

Pvent, proventriculus.

Phy, larval pharyngeal skeleton.

Rect, rectum.

SalD, salivary duct.

SalGl, salivary gland.

TMcl, transverse, intersegmental muscles.

Vent, ventriculus.

VMcl, latero-ventral longitudinal muscles of body wall.

VPhy, ventral wall of pharynx.

## PLATE 5

### *Rhagoletis pomonella*

A.—Puparium, lateral view.

B.—Puparium, dorsal view.

C.—Prepupa, or fourth-instar larva, exposed inside the puparium, 24 hours after formation of puparium.

D.—Pupa in early part of first or cryptocephalic stage, shedding the prepupal cuticle over the thorax, 43 hours after formation of puparium.

E.—The same as D, ventral view, but with puparial shell and larval pharynx removed, exposing a deep hole (*Atr*) in anterior end of pupa.

F.—Final phase of cryptocephalic pupa (*Pu*) inclosed in two envelopes, the puparial shell (*pm*) and the prepupal skin (*ppu*) now shed over entire body, but with larval linings of stomodeum, proctodeum, and tracheæ not yet cast out of body of pupa.

### EXPLANATION OF SYMBOLS

*An*, anus.

*ASp*, anterior larval spiracle.

*Atr*, atrium, anterior part of larval pharynx resulting from involution of original head of larva.

*Mth*, larval mouth.

*N*<sub>1</sub>, pronotum.

*N*<sub>2</sub>, Mesonotum.

*o*, horizontal cleavage line of puparium.

*p*, circular cleavage line of puparium.

*pm*, puparium.

*ppu*, prepupal larva (fourth larval instar, inside of puparium).

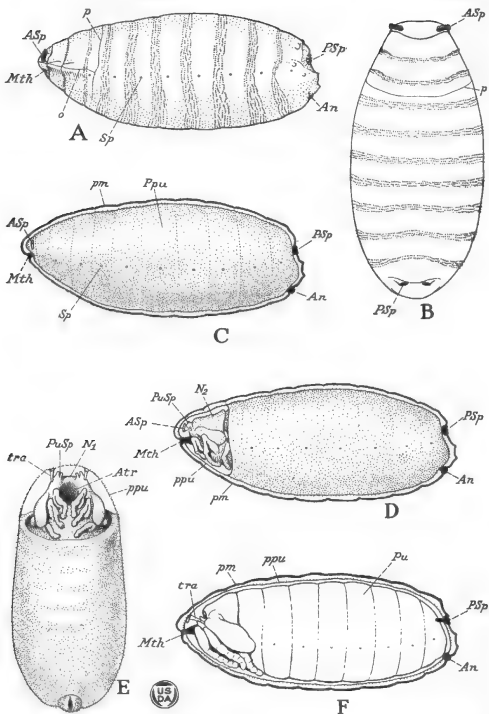
*PSP*, posterior larval spiracle.

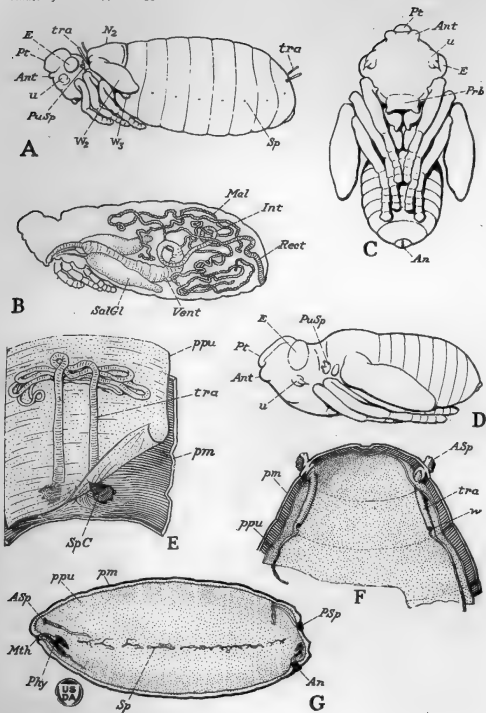
*Pu*, pupa.

*PuSp*, pupal dorsal spiracle of pronotum.

*Sp*, lateral spiracles, the adult system, connected with lower longitudinal tracheal trunks.

*tra*, tracheal linings of preceding instar.





## PLATE 6

### *Rhagoletis pomonella*

A.—Pupa in early part of second or phanerocephalic stage. Head everted, larval tracheal linings still retained.

B.—Pupal alimentary canal and salivary glands at period shown in A. (Compare with Plate 4, A.)

C.—Second phase of phanerocephalic pupa, the final pupal form, ventral aspect.

D.—Same as C, lateral aspect.

E.—Cast linings of dorsal tracheal trunks of larva connected with stigmatic chambers of posterior spiracles of puparium, showing usual arrangement against inner wall of molted prepupal cuticle enclosing the pupa.

F.—Inner surface of dorsal part of puparial cuticle through first three segments, and prepupal cuticular envelope of pupa, showing cast larval tracheal tubes still connected with anterior larval spiracles of puparium, and opening into pupal chamber by ruptures in second segment, thus establishing air passages to the pupa.

G.—Median, lengthwise section of puparium, and inner pupal envelope formed of cuticle of prepupal instar, pupa removed, showing cast linings of dorsal tracheal trunk connected with anterior and posterior larval spiracles of puparium, linings of lateral trunks and branches connected with lateral spiracles, and intima of pharynx and intestine connected with pupal envelopes at mouth and anus.

### EXPLANATION OF SYMBOLS

*An*, anus.

*Ant*, antennal lobes.

*ASp*, anterior larval spiracle.

*E*, compound eye.

*Int*, intestine.

*Mal*, Malpighian tubules.

*Mth*, larval mouth.

*N<sub>2</sub>*, mesonotum.

*Phy*, larval pharyngeal skeleton.

*pm*, puparium.

*ppu*, cuticle of prepupal larva.

*PSp*, posterior larval spiracle.

*Prb*, proboscis.

*Pt*, ptilinum.

*PuSp*, pupal dorsal spiracle of pronotum.

*Rect*, rectum.

*SalGl*, salivary gland.

*Sp*, lateral spiracles, the adult system, connected with lower longitudinal tracheal trunk.

*SpC*, stigmatic chamber.

*tra*, tracheal linings of preceding instar.

*u*, subocular lobe of pupa.

*Vent*, ventriculus.

*W<sub>2</sub>*, wing; *W<sub>3</sub>*, haltere.

# CHEMICAL AND BIOLOGICAL STUDIES WITH CYANAMID AND SOME OF ITS TRANSFORMATION PRODUCTS<sup>1</sup>

By K. D. JACOB, *Assistant Chemist*, F. E. ALLISON, *Soil Biochemist*, and J. M. BRAHAM, *Chief, Chemistry Division, Fixed Nitrogen Research Laboratory, United States Department of Agriculture*

## INTRODUCTION

In order for any nitrogenous material to be a satisfactory source of nitrogen for a wide variety of plants and under varying soil conditions it is necessary that the nitrogen be present either in the nitrate form or be readily attacked by the soil flora and converted into nitrates. While many plants can use ammonia and certain organic forms of nitrogen for their synthetic processes, most plants require at least a portion of their nitrogen in the form of nitrates. The investigations reported here were undertaken in order to obtain data on the rate at which cyanamid (calcium cyanamid) and its transformation products are broken down to ammonia and the latter oxidized to nitrates in the soil. In addition, the effect of dicyanodiamid and guanylurea on the nitrification of the soil organic matter or nitrogenous fertilizers which may be applied has been given consideration. The term ammonification is used here in the broader sense to include all of the decomposition processes from which ammonia is finally obtained. The term nitrification covers the oxidation of the ammonia to nitrates with nitrites as an intermediate product.

For convenience this paper has been divided into two parts; the first dealing with cyanamid and urea which under normal conditions are transformed into compounds which are suitable plant foods; the second with dicyanodiamid, guanylurea, guanidin, and biguanid nitrogen which are less desirable in soils. While it is probable that these latter compounds may serve as sources of nitrogen for plants in some instances, their value is questionable and some of them, particularly dicyanodiamid, are harmful.

In using the term transformation products of cyanamid to include the material discussed in Part II, it is not meant to imply that all of them are formed in the soil when cyanamid is applied. Whether or not this is true still remains to be shown. We do know that small amounts of dicyanodiamid are commonly formed and under unusually bad soil conditions the amount may be large, perhaps 25 per cent of the original nitrogen. There is little known as to the formation of salts of guanylurea, guanidin, and biguanid but it is logical to suppose that small percentages of these may appear under certain soil conditions. The uncertainty with regard to the appearance of these compounds does not necessarily detract from the value of a study of their behavior because they may be found in old samples of cyanamid or in fertilizer mixtures containing acid salts with which the cyanamid has reacted.

## PREVIOUS INVESTIGATIONS AND DISCUSSION

### CYANAMID AND UREA

A large number of laboratory and field studies have been reported which deal with the changes which cyanamid undergoes in the soil and the response of

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<sup>1</sup> Received for publication, Jan. 4, 1924.



various crops to the products produced. However, only a few of these which throw light on the nature of the cyanamid changes, particularly those resulting from biological action, are mentioned here. The work is discussed approximately in the order reported.

Löhnis (13)<sup>2</sup> stated that on laboratory culture media cyanamid was converted into ammonia by bacterial action in the same manner as that of urea but at a slower rate. Dicyanodiamid was also formed in the absence of microorganisms by the action of water. Perotti (22) obtained results quite similar to those reported by Löhnis.

Kappen (9) found that in loam soil the transformation of cyanamid was very rapid and only small percentages of dicyanodiamid were formed. He attributed the first poisonous effects on plants following top dressings of cyanamid on acid soils to cyanamid, the later effects to dicyanodiamid.

Müntz and Nottin (21) obtained a lower rate of nitrification of calcium cyanamid than of ammonium sulphate when used in relatively large amounts but found little difference at the lower rates. They attributed the retardation to the cyanamid, itself, and not to the caustic lime present. Likewise, Lipman and Brown (12) found that cyanamid was injurious to nitrifying bacteria.

Löhnis and Sabaschnikoff (16) stated that cyanamid is not converted to any appreciable extent into ammonia in sterile soils but microorganisms are necessary for the change. Contrary to the opinion of Löhnis, Ulpiani (26, 27) concluded from his studies that cyanamid is not converted into ammonia by bacteria, but easily changes into dicyanodiamid, urea, and other compounds. The dicyanodiamid and urea are both converted into ammonia. In a subsequent paper (15) Löhnis and Moll agreed with Ulpiani that there is no direct bacterial action on cyanamid, but that microorganisms are responsible for the formation of ammonia from the urea, first produced from cyanamid by soil colloids. On the other hand, Kappen (10) claimed to have found five species of fungi which readily attacked cyanamid.

Stutzer, Reis, and Söll (25) believed that the formation of ammonia from cyanamid was due chiefly to chemical action but, nevertheless, claimed that cyanamid but not dicyanodiamid can be utilized by microorganisms as a source of nitrogen. Brioux (2) found that in soil some cyanamid polymerizes into dicyanodiamid but that the latter is quickly converted into ammonia.

The importance of colloids was emphasized by several investigators, Henschel (7) having obtained a somewhat more rapid decomposition of cyanamid in dry sterilized soils and colloids than in the unsterilized. The presence of humus was of especial importance. Milo (20) studied the decomposition of cyanamid in soils and states that in heavy, strongly absorbent soils, high in colloidal matter urea is quickly formed by chemico-physical action. The urea is in turn changed to ammonium carbonate probably by microorganisms. In light soils the calcium cyanamid formed basic calcium cyanamid salts and free cyanamid. Ammonia was formed from these very slowly, thus giving time for dicyanodiamid formation. Löhnis (14) as a result of further studies, similar to those mentioned, agreed with Kappen that soil fungi are able to attack cyanamid directly but emphasized that normally cyanamid is changed into urea by means of colloids and that this is in turn changed into ammonia and nitrates by biological action.

Mazé and Lemoigne (19) found that a number of common soil bacteria are capable of converting cyanamid into urea. This change takes place rapidly in fertile soils, rich in humus, but slowly in acid soils. The urea is quickly changed to ammonia and nitrates.

Cowie (3) in a study of the chemistry of cyanamid changes in soil reported that cyanamid is readily changed to ammonia and nitrates in the absence of dicyanodiamid. When the latter compound is present ammonia accumulates

<sup>2</sup> Reference is made by number (*italic*) to "Literature cited," p. 68-69.

and nitrification is inhibited. In further work (4) with cyanamid he concluded that urea formation is the result of a purely chemical process and not biological. The breaking down of the urea is the result of the work of microorganisms. Cyanamid decomposition was more rapid in clay than in sandy soils.

The wide variations in the results reported by investigators who have studied cyanamid decomposition may be attributed largely to the differences in the methods of experimentation, including variations in the cyanamid and soils used. For instance, any experimentation with cyanamid containing appreciable quantities of dicyanodiamid or under conditions that favored its formation would undoubtedly greatly affect the conclusions reached by the investigator. This point will be considered more fully in the latter part of this report. However, the most recent investigations, as well as the observations of the writers, make it seem probable that under normal soil conditions cyanamid quickly changes into calcium acid cyanamid, then free cyanamid, urea, and ammonia. The ammonia is in turn oxidized to nitrites and nitrates. Dicyanodiamid is produced as a side reaction but only in small amounts, except under unfavorable soil conditions. This point has been considered in another publication (1) particularly in relation to the utilization of cyanamid by plants. The exact nature of the changes taking place, whether the result of chemical or biological action, is still disputed. However, it seems that the changes which result in the production of urea from cyanamid are largely, if not wholly, chemical and physical. Colloids are especially important in hastening these processes. The formation of ammonia from urea and its oxidation to nitrates is the result of the work of microorganisms.

#### DICYANODIAMID

In a study of dicyanodiamid De Grazia (6) failed to obtain nitrification. Perotti (23) studied the growth of bacteria on culture media containing dicyanodiamid and concluded that it is a good nitrogen food for many microorganisms. Ulpiani (26) reported that dicyanodiamid is slowly converted into ammonia. Loew (17) found that dicyanodiamid is not a good source of nitrogen for many kinds of soil bacteria. Löhnis (15) failed to secure any evidence that bacteria attack dicyanodiamid in either dilute or concentrated solutions. Stutzer (25) reported that dicyanodiamid can not be used by microorganisms, but in the soil is converted into ammonia by the chemical action of certain soil constituents. Reis (24) stated that certain microorganisms can use dicyanodiamid and also guanylurea and biguanid nitrogen in concentrations of less than one part per thousand of water. Brioux (2) found that if thoroughly mixed with the soil dicyanodiamid is quickly converted into ammonia and nitrified. Cowie (3) failed to obtain any evidence of nitrification of dicyanodiamid in soils even after several months. Furthermore, it was toxic to the nitrifying organisms and stopped the oxidation of ammonia in soils containing ammonium sulphate. It did not prevent the formation of ammonia from cyanamid or other forms of nitrogen but it did prevent the oxidation to nitrates, resulting in an ammonia accumulation. This extreme toxicity was limited to the nitrifying bacteria, the other soil organisms apparently being practically unaffected. Linter (11) did not find that any decomposition of dicyanodiamid occurred in soils during a period of several days.

The results of the investigations mentioned vary as to the readiness with which dicyanodiamid is attacked by bacteria. Where decomposition was obtained, some writers attribute it to purely chemical action, others to biological. No attempt was made in the work reported here to determine the agents responsible for the breaking down of the compound, but certainly there is no doubt but that the material does go over to ammonia. With the exception of the work of

Brioux, the previous investigations noted agree that dicyanodiamid does not nitrify. Using the ordinary applications of nitrogen and the short incubation period, the present work bears out this statement, but using 12.7 mgm. of nitrogen per 250 gm. of soil a nitrification of 38.6 per cent was obtained after 40 weeks. It is logical to suppose that nitrification should eventually take place since ammonia is formed fairly readily. The extreme toxicity of the dicyanodiamid for the nitrifying organisms prevents the normal oxidation process until most of the material has gone over to ammonia and the bacteria have recovered. It is this property of dicyanodiamid which is largely responsible for its injurious effect in fertilizers, but doubtless it is also directly toxic to many plants.

#### GUANYLUREA

The experimental work with guanylurea which has been reported is very limited and consists for the most part of plant culture studies. Since such studies are not directly related to the work here reported no references are given. It is sufficient to state that for the most part guanylurea has not served as a satisfactory plant food and in some cases has been reported as toxic. The behavior of the material in laboratory studies would lead us to expect just such a response. The material ammonified very slowly, and hence nitrate formation was limited. Where guanylurea sulphate was applied with ammonium sulphate the nitrification of the latter was inhibited for some time, but the toxic effect was decidedly less than with dicyanodiamid. However, such large quantities of guanylurea sulphate were required to appreciably affect the oxidation of ammonium sulphate under laboratory conditions that it seems highly improbable that the material would ever be present in fertilizers in quantities sufficient to be injurious to field crops. Nitrogen present in the guanylurea form should be considered as inert material without fertilizer value.

#### GUANIDIN

Guanidin has given quite variable results when used as a fertilizer. In most cases the nitrate salt has been used and any increases in growth have been attributed to the nitrate rather than to the guanidin. Again, the nitrification experiments would lead us to expect the material to be an unsatisfactory source of nitrogen since it prevented nitrification for several weeks after application. Later it was quite rapidly converted into nitrates. If used on a soil containing sufficient nitrate nitrogen to supply the needs of the plants for the first four to eight weeks until the guanidin nitrogen had become available then the salts might act as good fertilizers. This also explains why in some cases guanidin salts gave better results on the second crop than on the first.

#### BIGUANID

Laboratory or field studies with biguanid nitrogen are very limited and its value still remains to be established. Reis (24) found that in the laboratory the compound could be used by microorganisms if the concentration was sufficiently low. In the present nitrification studies a maximum conversion of 9.7 per cent was obtained after 50 days. This figure is so low that the slight increase in nitrates may have come from the soil organic matter rather than from the biguanid nitrogen. Again, the increase may have represented merely the injury which the biguanid caused to the organisms which use nitrate nitrogen.

#### PLAN OF THE WORK

The experiments, which consisted chiefly of ammonification and nitrification studies, were conducted in the laboratory, using either 100 or 250 gm. of air-dried

soil in 200 or 300 cc. beakers, to which were added the various materials being studied. The soil used for all of the experiments was a Susquehanna loam, well supplied with organic matter and quite productive. It had been in constant cultivation for about 15 years. As shown by the experimental results, it contained a sufficient supply of basic elements to show a high nitrifying efficiency even without additions of calcium carbonate.

In preparing mixtures, usually the required amount of the dry salt or fertilizer was thoroughly mixed with the soil sample by means of a small aluminum shaker of the type commonly used in soda fountains. In a few cases the soluble salts were added in solution. The moisture content was brought up to the optimum of 21 cc. per 100 gm. of soil, except in one instance where this was made the variable factor. The water lost by evaporation was replaced at intervals of four or five days. During the incubation period the soils were maintained at room temperature, usually about 20° to 22° C., except where the temperature was made the variable factor.

The analyses of the nitrogenous compounds used is given in Table I. The untreated cyanamid was used unless otherwise stated.

ANALYTICAL METHODS

DETERMINATION OF AMMONIA NITROGEN

To a 25-gm. sample of soil were added 50 cc. of a solution of sodium carbonate and sodium chlorid (108 gm. Na<sub>2</sub>CO<sub>3</sub> and 150 gm. NaCl per liter) and about 1 cc. of paraffin oil. The mixture was then aerated for six hours in the apparatus devised by Matthews (18). The ammonia evolved was absorbed in N/20 sulphuric acid and the excess titrated with N/20 sodium hydroxid, using methyl red as an indicator.

TABLE I.—Total nitrogen content of materials used

Total nitrogen (per cent)		Total nitrogen (per cent)	
Cyanamid, hydrated and oiled	18. 32	Guanylurea sulphate	34. 92
Cyanamid, untreated	20. 61	Guanidin nitrate	45. 44
Urea	46. 44	Guanidin carbonate	46. 62
Ammonium sulphate	20. 90	Nitro guanidin	53. 84
Dicyanodiamid	66. 31	Biguanid nitrate	51. 00

TABLE II.—Determination of ammonia by the aeration method

Material	Mgm. N added	Nitrogen recovered as ammonia							
		1.5 hours		3 hours		5 hours		6 hours	
		NH <sub>3</sub> mgm. N	Aver- age	NH <sub>3</sub> mgm. N	Aver- age	NH <sub>3</sub> mgm. N	Aver- age	NH <sub>3</sub> mgm. N	Aver- age
Blank	0. 00			0. 04				0. 04	
Soil	. 00			. 19		0. 19		. 19	
	. 00			. 17	0. 18	. 18	0. 18	. 21	0. 20
Ammonium sulphate from solution	10. 40	9. 07		10. 37				10. 38	
	10. 40	9. 73	9. 40	10. 40	10. 38			10. 38	10. 38
Ammonium sulphate from soil	10. 40			9. 64		10. 21		10. 27	
	10. 40			9. 89	9. 76	10. 17	10. 19	10. 22	10. 24
Dicyanodiamid	2. 54							. 005	

The accuracy of the method is indicated by the results of the preliminary experiments reported in Table II. Almost complete recovery of the ammonia in ammonium sulphate was secured after aerating three hours. In the presence of soil it was more difficult to drive off the ammonia, 93.9 per cent being recovered in three hours and 98.5 per cent after six hours. When a dicyanodiamid solution was aerated the recovery as ammonia was 0.18 per cent. This small amount is easily within experimental error when dealing with such small quantities of nitrogen.

#### DETERMINATION OF NITRATE NITROGEN

Two methods of determining nitrates were used in this work, namely, the reduction and the colorimetric methods.

##### REDUCTION METHOD

The determination of nitrate nitrogen by the reduction method, using Devarda's alloy, is now generally considered as the most satisfactory for most purposes. However, in the presence of undecomposed cyanamid, dicyanodiamid, urea, or guanylurea the reduction method as ordinarily carried out gives high results. This is because these compounds, when boiled with weak alkali, slowly decompose with the evolution of ammonia. A modification of the method to avoid the difficulties was devised and has been reported elsewhere (8). The modified procedure was used in this work wherever the reduction method was employed.

##### COLORIMETRIC METHOD

The phenol-disulphonic acid method of determining nitrates in soil solutions has been practically set aside in recent years because there are numerous salts and organic materials which interfere in the color production or particular shade of color. Regardless of these objections to an indiscriminate use of the method it is nevertheless recognized that under certain conditions the method is quite accurate, and much shorter than the reduction method.

A comparison between the modified reduction method and the phenol-disulphonic acid method was made during the course of these investigations on samples of soil containing cyanamid and its decomposition products. For the particular soil used the results were in quite close agreement and the compounds which require elimination in the case of the reduction method did not interfere in the colorimetric method. For small quantities of nitrate nitrogen the latter method is more accurate, and since it is shorter it was used in obtaining a portion of the results reported in this paper. Where this method was used the fact is mentioned, otherwise the modified reduction method was used.

#### DETERMINATION OF COMBINED CYANAMID AND DICYANODIAMID NITROGEN

The method of Brioux was used for the determination of cyanamid and dicyanodiamid nitrogen. To 50 cc. aliquots of soil extract were added 10 cc. each of 5 and 10 per cent solutions of silver nitrate and potassium hydroxid, respectively. It was found necessary to wash the precipitates 12 or 13 times with distilled water in order to remove all traces of ammonia. The total nitrogen in the precipitate was then determined by the usual Kjeldahl method.

#### DETERMINATION OF UREA NITROGEN

The short urease method as developed at this laboratory by Fox and Geldard (5) was used for the determination of urea. This consists in adding to a 50 cc.

aliquot of soil extract 10 cc. of a freshly prepared neutral 2 per cent water extract of Jack-bean flour. After standing for one hour at room temperature the solution is made acid with a measured excess of standard acid, aerated for 5 minutes to drive off carbon dioxide and titrated. The acid used up is a measure of the ammonia formed from the urea. Blanks on soil extract alone showed no interference in this method.

## EXPERIMENTAL RESULTS

### PART I.—AMMONIFICATION AND NITRIFICATION OF CYANAMID AND UREA

#### COMPARATIVE RATES OF NITRIFICATION OF CYANAMID, UREA, AND AMMONIUM SULPHATE

The soil used for these experiments was obtained fresh, then sieved and air-dried. An analysis of the dry soil showed 0.56 mgm. of nitrate nitrogen per

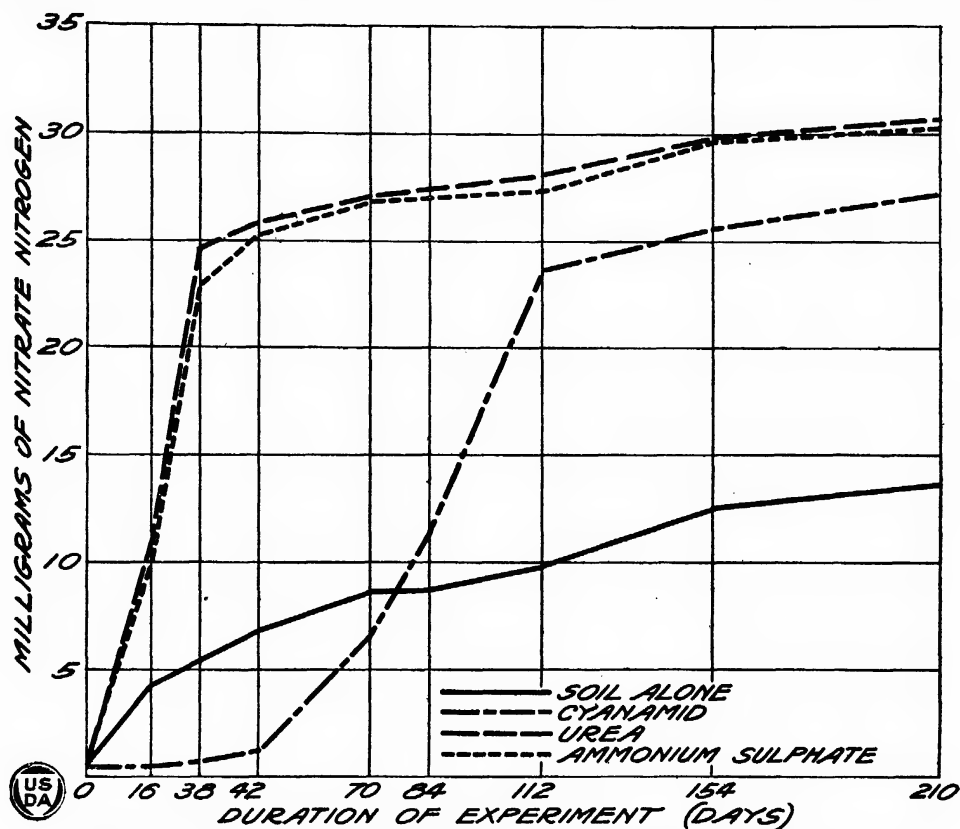


FIG. 1.—Diagram showing the rates of nitrification of cyanamid, urea, and ammonium sulphate.

100 gm. of soil. One gm. of powdered calcium carbonate was added to each 100 gm. of soil in order to insure neutrality. The figures given in Table III are the averages of duplicate determinations.

Considering the results in Table III, which are shown diagrammatically in figure 1, it will be observed that there was no very great difference between the rates of nitrification of ammonium sulphate and urea, the slight difference being in favor of the latter material. The maximum conversion of 87.8 per cent for ammonium sulphate was reached in 42 days, while with urea the maximum, 91.9 per cent, was reached in 28 days.

Cyanamid failed to show any nitrification during the first 70 days but actually depressed the nitrate formation from the soil organic matter. At the end of 84 days there was a 12.77 per cent conversion and after 112 days the maximum of only 66.65 per cent was reached. From subsequent experiments (Table IV) with the same or larger quantities of cyanamid it appears probable that the presence of the calcium carbonate had a deleterious effect upon the rate of nitrification of this material.

#### AMMONIFICATION AND NITRIFICATION OF VARYING QUANTITIES OF TREATED AND UNTREATED CYANAMID

These experiments were made to determine whether hydrating and oiling cyanamid has any effect upon its rate of decomposition and nitrification and hence upon its value as a fertilizer. The treatment given the cyanamid was the same as ordinarily used for commercial cyanamid and consisted of hydration with 8 per cent water and the addition of 3.5 per cent of mineral oil.

It was also desired to determine the maximum quantity of cyanamid which may be used without seriously affecting nitrification. Since the soil showed a high nitrifying efficiency without the addition of calcium carbonate none of the latter material was used.

In making the analyses, if urea was found to be present two samples of soil were analyzed, one for ammonia, nitrate, and urea and the other for combined cyanamid and dicyanodiamid. Only a qualitative test was made for cyanamid because of the great difficulty experienced in washing the silver precipitate free of all traces of ammonia. When urea was absent all of the analyses were made on one sample of soil. The results are given in Table IV and in part in figure 2. The data used in plotting the graph were those secured with oiled and hydrated cyanamid.

TABLE III.—Comparison of rates of nitrification of cyanamid, urea, and ammonium sulphate

Treatment (20.9 mgm. N per 100 gm. soil)	16 days		28 days		42 days		70 days	
	Nitrate mgm. N	Increase over control mgm.	Nitrate mgm. N	Increase over control mgm.	Nitrate mgm. N	Increase over control mgm.	Nitrate mgm. N	Increase over control mgm.
Soil alone.....	4.31	-----	5.51	-----	6.91	-----	8.65	-----
Cyanamid.....	4.48	0.00	.74	0.00	1.23	0.00	6.70	0.00
Urea.....	11.31	7.00	24.71	19.20	25.86	18.95	27.13	18.48
Ammonium sulphate.	10.64	6.33	23.01	17.50	25.26	18.35	26.88	18.23

Treatment (20.9 mgm. N per 100 gm. soil)	84 days		112 days		154 days		210 days	
	Nitrate mgm. N	Increase over control mgm.	Nitrate mgm. N	Increase over control mgm.	Nitrate mgm. N	Increase over control mgm.	Nitrate mgm. N	Increase over control mgm.
Soil alone.....	8.66	-----	9.83	-----	12.50	-----	13.75	-----
Cyanamid.....	11.33	2.67	23.76	13.93	25.58	13.08	27.28	13.53
Urea.....	-----	-----	28.11	18.28	29.85	17.35	30.78	17.03
Ammonium sulphate.	-----	-----	27.39	17.56	29.65	17.15	30.27	16.52

<sup>a</sup> Qualitative tests showed absence of cyanamid nitrogen.



The ammonia and nitrate production from cyanamid, particularly nitrate, was dependent upon the rate of application. With the smallest applications nitrification was complete within 19 days while with the largest amount, namely, 104.4 mgm. N per 250 gm. of soil, nitrification was only about 50 per cent complete in 200 days. In such cases the cyanamid nitrogen remained in the soil largely in the form of cyanamid, dicyanodiamid, and ammonia. In the case of the small applications of cyanamid more than a 100 per cent recovery of the

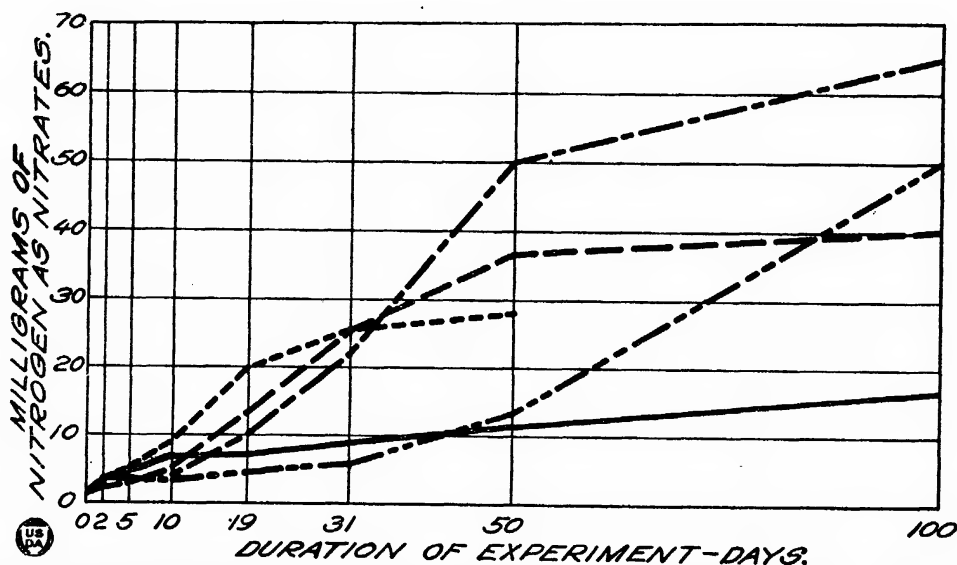
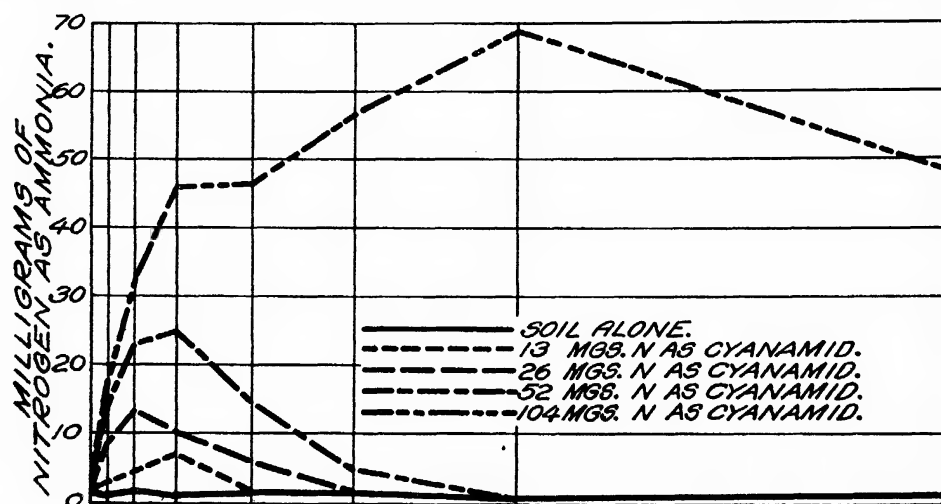


FIG. 2.—Diagram showing the rates of ammonification and nitrification of different quantities of hydrated and oiled cyanamid

added nitrogen was obtained in some instances. This may have been due to a stimulating action which small quantities of cyanamid may possibly have had upon the nitrifying organisms, causing a more rapid nitrification of the organic soil nitrogen than takes place in the soil containing no cyanamid. However, no great significance should be attached to this since, as previously stated, the reduction method is not very accurate for the determination of very small amounts of nitrates. In these experiments 200 gm. of soil were extracted with 500 cc. of

water and 200 cc. aliquots distilled. Each distillation, therefore, represented only 2 to 3 mgm. of the original cyanamid nitrogen in the case of the smallest applications.

TABLE IV.—Ammonification and nitrification of varying quantities of treated and untreated cyanamid

Treatment	Incuba- tion period	Ammonia mgm. N	Nitrates mgm. N	Recovery of N		
				Ammonia mgm. N	Nitrates mgm. N	Total mgm. N
	Days					
Soil alone.....	0	1.52	1.64			
Do.....	2	.08	3.39			
Do.....	5	1.07	4.55			
Do.....	10	.84	6.36			
Do.....	19	1.20	6.72			
Do.....	31	1.33	8.53			
Do.....	50	.65	11.36			
Do.....	100	1.21	16.01			
Do.....	150	.79	19.29			
Do.....	200	.26	20.78			

NITROGEN ADDED—6.52 MGM. PER 250 GM. SOIL

Cyanamid treated.....	10	1.52	9.32	0.68	2.96	3.64
Do.....	19	1.48	14.38	.28	7.66	7.94
Do.....	31	.74	16.16	— .59	7.63	7.04
Do.....	50	1.15	19.16	.50	7.80	8.30
Cyanamid untreated.....	10	3.18	9.17	2.34	2.81	5.15
Do.....	19	1.21	16.06	.01	9.34	9.35
Do.....	31	.65	18.93	— .68	10.40	9.72
Do.....	50	.85	21.35	.20	9.99	10.19

NITROGEN ADDED—13.04 MGM. PER 250 GM. SOIL

Cyanamid treated.....	10	6.89	8.75	6.05	2.39	8.44
Do.....	19	1.38	19.10	.18	12.38	12.56
Do.....	31	.74	24.77	— .59	16.24	15.65
Do.....	50	1.00	27.72	.35	16.36	16.71
Cyanamid untreated.....	10	8.22	8.83	7.38	2.47	9.85
Do.....	19	1.34	20.04	.14	13.32	13.46
Do.....	31	1.02	24.83	— .31	16.30	15.99
Do.....	50	1.02	26.51	.37	15.15	15.52

NITROGEN ADDED—26.08 MGM. PER 250 GM. SOIL

Treatment	Incuba- tion period	Cyan- amid and di- cyan- amid mgm. N	Test for cyanamid	Urea mgm. N	Am- monia mgm. N	Ni- trates mgm. N	Recovery of N		
							Am- monia mgm. N	Ni- trates mgm. N	Total mgm. N
	Days								
Cyanamid treated.....	2	8.48	Present	0.37...	8.22	2.26	7.42	—1.13	15.14
Do.....	5	1.87	None	None	13.18	2.61	12.11	—1.94	12.04
Do.....	10	.89	do	do	10.47	4.72	9.63	—1.64	8.88
Do.....	19	.80	do	do	6.52	12.26	5.32	5.54	11.66
Do.....	31	.59	do	do	1.39	24.77	.06	16.24	16.89
Do.....	50	.56	do	do	.32	36.27	— .33	24.91	25.14
Do.....	100	.00	do	do	1.21	40.03	.00	24.02	24.02
Cyanamid untreated.....	10				12.90	8.85	12.06	2.49	14.55
Do.....	19				4.23	21.32	3.03	14.60	17.63
Do.....	31				1.02	34.45	— .31	25.92	25.61
Do.....	50				1.10	38.21	.45	26.85	27.30
Do.....	100				1.21	43.75	.00	27.74	27.74

TABLE IV.—Ammonification and nitrification of varying quantities of treated and untreated cyanamid—Continued

NITROGEN ADDED—52.2 MGM. PER 250 GM. SOIL

Treatment	Incubation period	Cyanamid and dicyanamid mgm. N	Test for cyanamid	Urea mgm. N	Ammonia mgm. N	Nitrates mgm. N	Recovery of N		
							Ammonia mgm. N	Nitrates mgm. N	Total mgm. N
	Days								
Cyanamid treated	2	24.76	Present	1.73	13.86	2.38	13.06	−1.01	38.54
Do	5	8.23	Trace	Trace	22.12	2.53	21.05	−2.02	27.26
Do	10	3.75	None	None	24.42	3.94	23.58	−2.42	24.91
Do	19	2.49	do	do	14.10	9.59	12.90	2.87	18.26
Do	31	2.77	do	do	4.96	21.43	3.63	12.90	19.30
Do	50	.75	do	do	1.21	49.07	.56	37.71	39.02
Do	100	.00	do	do	1.22	64.35	.01	48.34	48.35
Do	150	.00	do	do	1.00	63.75	.21	44.46	44.67
Cyanamid untreated	2	23.14	Present	1.59	14.28	2.43	13.48	−.96	37.25
Do	5	6.48	None	Trace	25.75	2.36	24.68	−2.19	28.97
Do	10	3.13	None	None	27.40	3.70	26.56	−2.66	27.03
Do	19	.62	do	do	12.63	12.41	11.43	5.69	17.74
Do	31	1.28	do	do	11.03	21.10	9.70	12.57	23.55
Do	50	.72	do	do	.70	54.65	.05	43.29	44.06
Do	100	.00	do	do	1.23	64.52	−.02	48.51	48.53
Do	150	.00	do	do	1.26	66.69	.47	47.40	47.87

NITROGEN ADDED—104.4 MGM. PER 250 GM. SOIL

Cyanamid treated	2	57.92	Present	5.59	17.68	2.95	16.88	−0.44	79.95
Do	5	32.77	do	1.47	31.63	2.80	30.56	−1.75	63.05
Do	10	14.53	None	1.11	45.62	3.02	44.78	−3.34	57.08
Do	19	9.84	do	1.20	46.35	4.21	45.15	−2.51	53.68
Do	31	9.37	do	None	56.60	5.58	55.27	−2.95	61.69
Do	50	9.04	do	do	68.43	13.17	67.78	1.81	78.63
Do	100	3.61	do	do	48.44	49.94	47.25	33.93	84.77
Do	150	4.20	do	do	41.87	56.78	41.08	37.49	82.77
Do	200	2.65	do	do	39.52	69.47	39.26	48.69	90.60
Cyanamid untreated	10				51.08	3.62	50.24	−2.74	47.50
Do	19				39.25	7.67	38.05	.95	39.00
Do	31				60.20	11.36	58.87	2.83	61.70
Do	50				41.78	28.48	41.13	17.12	58.25
Do	100				25.07	59.92	23.86	43.91	67.77
Do	150				42.93	54.91	42.14	35.62	77.76
Do	200				17.68	85.78	17.42	65.00	82.42

The test for cyanamid showed none present at the end of 10 days and with the smaller concentrations of cyanamid none could be found after 5 days. The figures for the determination of combined cyanamid and dicyanodiamid nitrogen showed a considerable amount present in these two forms even after 10 days with the larger applications. Since qualitative tests showed cyanamid absent after this length of time we would naturally assume that all of the nitrogen precipitated by the method of Brioux was dicyanodiamid. However, silver nitrate precipitates guanylurea, guanidin and other decomposition products of cyanamid which may possibly have been present. Whether such compounds are formed in appreciable amounts in normal soils still remains to be shown.

Urea determinations showed only very small quantities present at any time although it is undoubtedly an intermediate product in the decomposition of cyanamid in the soil. Ammonia formation from urea in the presence of a mixed flora of microorganisms takes place so rapidly that no accumulation can occur.

The two samples of cyanamid, one oiled and hydrated and the other untreated, nitrified at about the same rates, where used in the smaller concentrations. At the heavier rates the latter appeared to be somewhat better than the former. Shortly after these experiments were started the hydrated and oiled cyanamid used in the mixtures was again analyzed for its various forms of nitrogen. It was found that the dicyanodiamid nitrogen had increased considerably over that found at the previous analysis, which was made about two months before the

experiments were started. The better results obtained with the untreated as compared with the maximum hydrated and oiled material may be in part attributed to the presence of this dicyanodiamid in the latter. At no time could any dicyanodiamid be found in the original sample of untreated cyanamid.

#### THE DECOMPOSITION OF UREA IN SOIL

This experiment was conducted in order to obtain data in addition to that already given in Table III on the rate of ammonification and nitrification of urea. The procedure followed was the same as in the earlier experiment except that no calcium carbonate was used. Preliminary experiments on the recovery of known quantities of urea from soil immediately after the addition gave an average of 97.63 per cent. The failure to recover all of the added urea may have been due to the rapidity with which this compound is decomposed to ammonia.

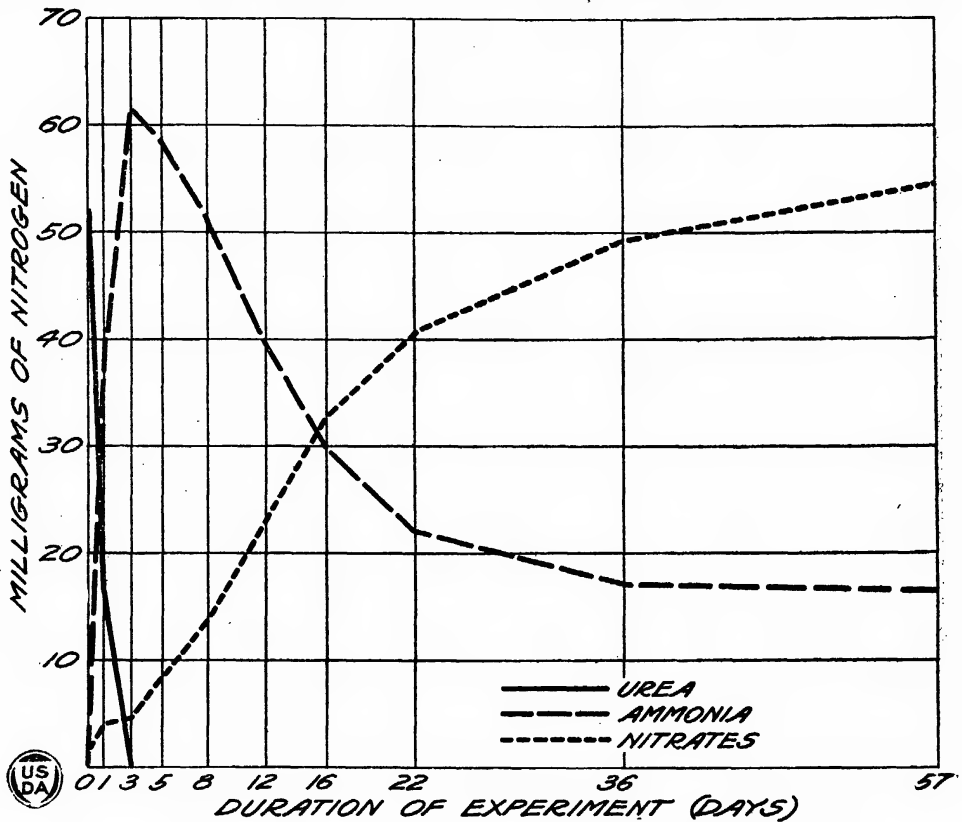


FIG. 3.—Diagram showing the rates of ammonification and nitrification of urea

in the soil. Further experiments to determine if urea is broken up in the aeration method for ammonia gave negative results.

The experimental results are given in Table V and figure 3. The results given for ammonia and nitrate nitrogen are the average of two determinations while those for urea are the average of three analyses, all made on the same sample of soil.

The conversion of urea into ammonia took place very rapidly, 65 per cent of the urea having disappeared within 24 hours while at the end of 3 days no trace of undecomposed urea could be found. A comparison of the results given in Table V with those in Table III shows a maximum nitrification of 74.6 per cent after 57 days in the former case and 91.8 per cent in 28 days in the latter. This wide variation is due to the marked stimulation of nitrate formation by the presence of calcium carbonate. It will be remembered that exactly opposite results were obtained in the case of cyanamid.

The importance of the manner in which the percentage nitrification is determined needs to be emphasized. There is no satisfactory method of measuring the actual per cent of the added nitrogen converted into nitrates in soil containing a mixed biological flora. All that is done is to determine the nitrates which accumulate over any given period. While the agents of decomposition are converting the nitrogen to ammonia, which in turn is oxidized to nitrites and nitrates, there are also numerous other organisms which are using the nitrates for their own growth. In this case the nitrates are usually converted into organic forms but under certain conditions may be merely denitrified. The soil conditions are, therefore, extremely important in determining the maximum nitrate accumulation at any given time. The more favorable the conditions for nitrate formation the quicker the maximum nitrate accumulation and usually the higher the figures. This is due chiefly to the fact that less time is available for the organisms, other than nitrifiers, to utilize the nitrate nitrogen. Where conditions permit only a slow rate of nitrification the nitrates may be utilized almost as fast as formed. The nitrogen thus converted into the organic form is again nitrified when the organisms die. Finally, an equilibrium point is reached after a long period where nitrate formation and nitrate utilization practically balance under a constant set of conditions. The nitrate nitrogen present at such a point is considerably lower than the maximum nitrate accumulation.

TABLE V.—Ammonification and nitrification of urea

Incubation period	Soil alone 250 gm.		Urea 52.25 mgm. N per 250 gm. soil					
	Ammonia mgm. N	Nitrates mgm. N	Urea mgm. N	Ammonia mgm. N	Nitrates mgm. N	Recovery of N		
						Ammonia mgm. N	Nitrates mgm. N	Total, including urea mgm. N
Days*								
0.....	1.88	1.63	52.25	1.88	1.63			
1.....	3.40	2.40	17.92	37.73	4.08	34.33	1.68	53.93
3.....	3.81	3.80	.00	61.81	4.73	58.00	.93	58.93
5.....	3.26	5.43	.00	58.70	8.23	55.44	2.80	58.24
8.....	2.81	7.85	.00	51.54	13.82	48.73	5.97	54.70
12.....	1.88	10.01	.00	40.02	23.39	38.14	13.38	51.52
16.....	.11	9.26	.00	29.61	32.53	29.50	23.27	52.77
22.....	.47	10.09	.00	22.31	40.65	21.84	30.56	52.40
36.....	1.12	12.08	.00	17.37	49.00	16.25	36.92	53.17
57.....	1.30	15.57	.00	16.53	54.55	15.23	38.98	54.21

The necessity for having a neutral or basic soil reaction in order to secure a rapid formation and accumulation of nitrates is very important in this connection. As nitric acid is formed, available basic elements must be present for the formation of neutral salts; otherwise further nitrification is practically prohibited. At the same time the growth of soil fungi, which prefer an acid medium, is favored and these organisms help further to decrease the nitrate supply. The failure of calcium carbonate to favor the nitrification of cyanamid, as previously mentioned, is an exception, but this is thought to be due to the increase in dicyanodiamid under such conditions.

EFFECT OF MOISTURE UPON THE NITRIFICATION OF CYANAMID AND UREA

The use of cyanamid as a fertilizer under field conditions has shown quite variable results apparently due largely to seasonal conditions in many instances. For instance, it has been stated that a wet period in the early summer retards the nitrification of cyanamid and hence poor responses from the use of the material are secured. Again, it has been claimed that cyanamid should be applied only

during the cool months of the year since a hot summer, which is usually accompanied by a drought, favors the formation of dicyanodiamid rather than nitrates. In order to secure more accurate data upon these points the two experiments which follow were made.

The methods were the same as previously given except that only 100-gm. samples of soil were used. Urea was selected as the standard for comparison

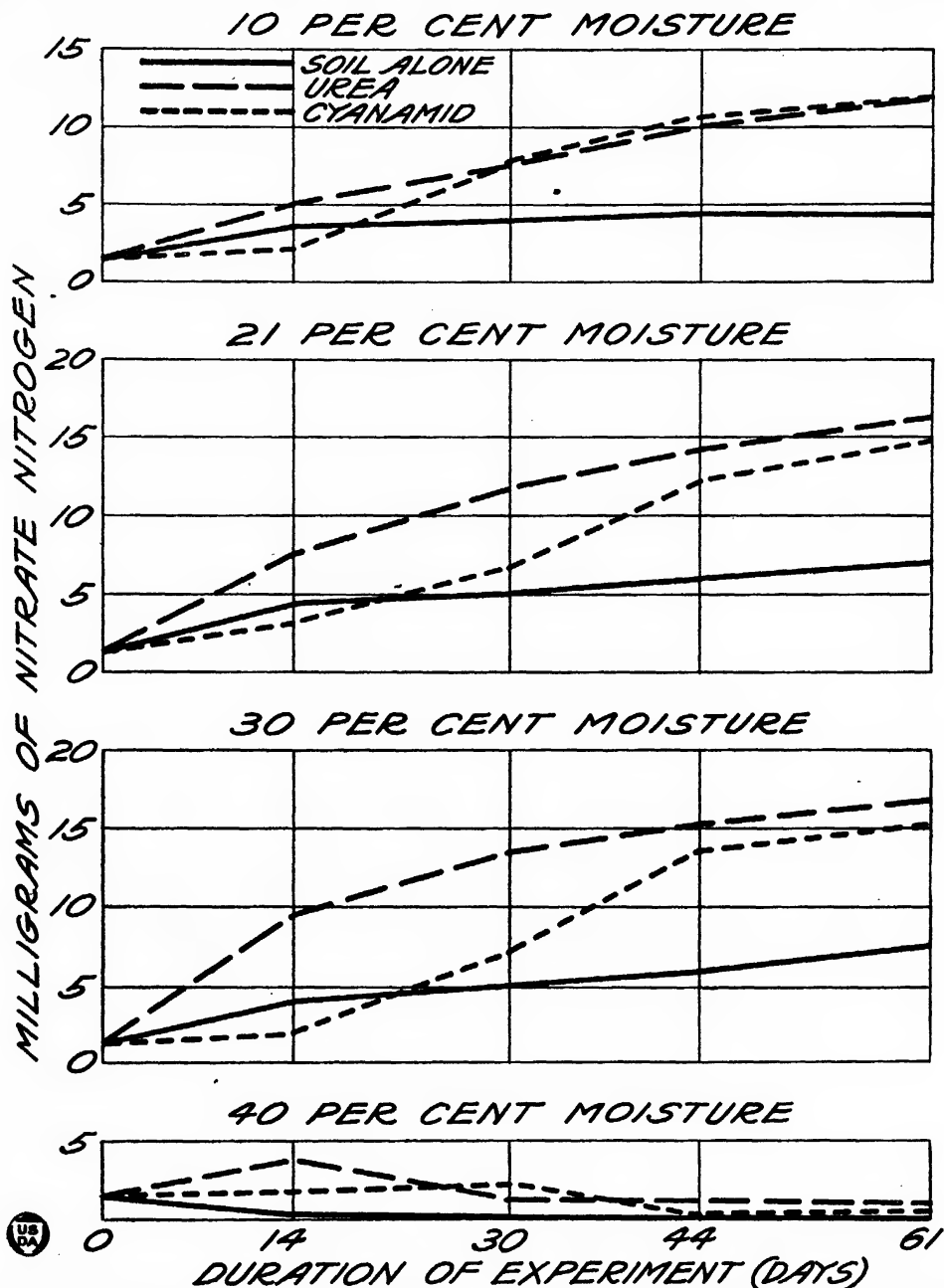


FIG. 4.—Diagram showing the rate of nitrification of cyanamid and urea in presence of different percentages of moisture

instead of ammonium sulphate. The soils were kept at room temperature during the incubation period. The data obtained by the colorimetric method, are given in Table VI and figure 4.

It will be noted that the per cent nitrification of cyanamid in the soil containing 10 per cent moisture is as high as that of urea under similar conditions

with the exception of the first incubation periods. This initial retardation is characteristic of cyanamid and occurs even under the most favorable conditions. In the presence of 21 and 30 per cent of water urea nitrified more rapidly in all cases than did cyanamid and even at the end of two months the nitrate accumulation was still higher in the case of urea. It should be noted that nitrification was the highest with both materials in the presence of 30 per cent of moisture which corresponds to nearly three-fourths of saturation. At half saturation (21 per cent), which is usually considered the optimum, the nitrate accumulation was appreciably below the figures for 30 per cent moisture. At 40 per cent moisture, which is near the saturation point, nitrification was negligible. In fact, there was usually a decrease of nitrates with an increase in the length of the incubation period. This may have been either the result of denitrification or utilization of the nitrates by microorganisms.

TABLE VI.—Nitrate formation from cyanamid and urea in soils containing different percentages of moisture

10 PER CENT MOISTURE					
Incubation period	Soil alone nitrates mgm. N	Urea 10 mgm. N per 100 gm. soil—		Cyanamid 10 mgm. N per 100 gm. soil—	
		Nitrates mgm. N	Recovery of N as nitrates mgm. N	Nitrates mgm. N	Recovery of N as nitrates mgm. N
Days					
14	3.57	5.15	1.58	2.53	−1.04
30	4.00	7.95	3.95	7.73	3.73
44	4.47	10.17	5.70	10.63	6.16
61	4.38	11.94	7.56	12.07	7.69

21 PER CENT MOISTURE					
14	4.65	7.72	3.07	3.50	−1.15
30	5.27	11.94	6.67	6.87	1.60
44	6.21	14.37	8.16	12.27	6.06
61	7.37	16.21	8.84	14.77	7.40

30 PER CENT MOISTURE					
14	4.16	9.54	5.38	2.23	−1.93
30	5.25	13.61	8.36	7.35	2.10
44	6.00	15.95	9.95	13.61	7.61
61	7.73	17.16	9.43	15.60	7.87

40 PER CENT MOISTURE					
14	0.38	3.81	3.43	(a)	(a)
30	.27	1.21	.94	2.42	2.15
44	.12	1.29	1.17	.35	.23
61	.00	1.08	1.08	.68	.68

<sup>a</sup> Lost.

EFFECT OF TEMPERATURE UPON THE NITRIFICATION OF CYANAMID AND UREA

This study of the nitrification of cyanamid was made according to the usual methods, using four different temperatures. One set of samples was kept in the open at a temperature varying from −9 to 18° C. Another series was maintained at a room temperature of 15 to 20° C. The remaining two sets of samples were kept at constant temperatures of 30° and 38.5° C. in incubators. The nitrate determinations, which were made by the colorimetric method, are given in Table VII. These data are plotted in figure 5.

The rate of nitrate formation at the lowest temperature was exceedingly slow in the case of the soil alone and also in the presence of urea. Even after 42 days, only about 10 per cent of the added nitrogen was recovered as nitrate nitrogen. With the equivalent application of cyanamid no nitrification occurred and even the nitrification of the soil organic matter was prevented. At room temperature

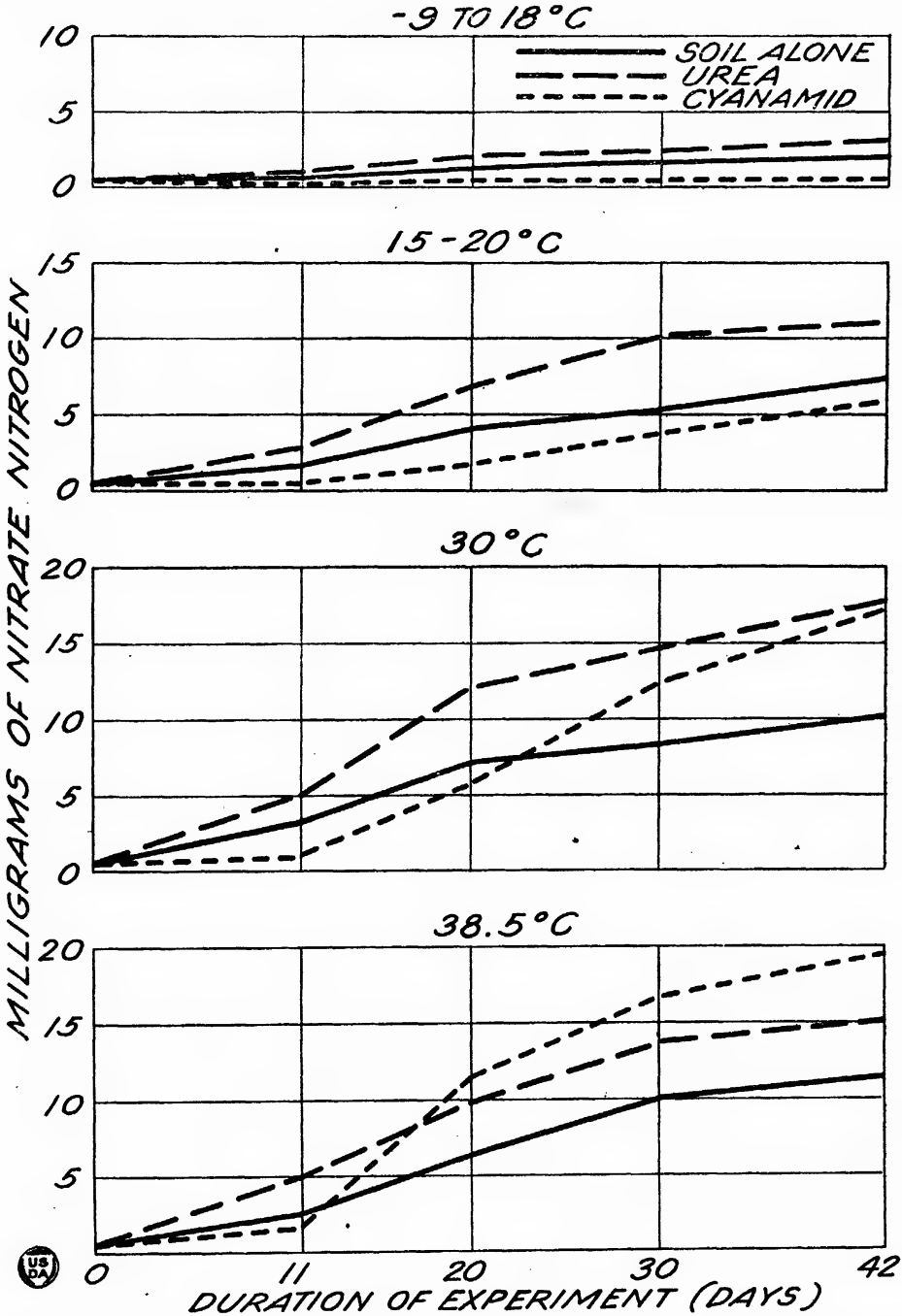


FIG. 5.—Diagram showing the rate of nitrification of cyanamid and urea at different temperatures

the urea nitrified to the extent of 49 per cent after one month but even after 42 days none of the cyanamid nitrogen was recovered as nitrate. In this experiment the same sample of cyanamid and the same soil type was used as in the previous experiments but the nitrification was poor. This may be attributed to the difference in the room temperature during the winter and summer months.



Nitrification of urea was the highest at 30° C., at which temperature 74 per cent of the added nitrogen was found as nitrate nitrogen. Under similar conditions cyanamid showed 69 per cent nitrification. At this temperature cyanamid retarded nitrification for 20 days but later was oxidized quite rapidly. At the highest temperature of 38.5° C. the nitrate accumulation from urea was considerably less than at 30° C.; but in the case of cyanamid was exceptionally good. The maximum nitrate accumulation for the entire experiment, which was 79.6 per cent, was obtained at this temperature. Under the same conditions urea gave 36.7 per cent.

TABLE VII.—*Nitrate formation from cyanamid and urea in soils maintained at different temperatures*

—9 TO 18° C.

Incubation period  <i>Days</i>	Soil alone nitrates mgm. N	Urea 10 mgm. N per 100 gm. soil—		Cyanamid 10 mgm. N per 100 gm. soil—	
		Nitrates mgm. N	Recovery of N as nitrates mgm. N	Nitrates mgm. N	Recovery of N as nitrates mgm. N
11.....	0.69	0.95	0.26	0.37	—0.32
20.....	1.36	2.06	.70	.50	— .86
30.....	1.56	2.40	.84	.54	—1.02
42.....	2.37	3.38	1.01	.73	—1.64

15 TO 20° C.

11.....	1.84	2.72	0.88	0.55	—1.29
20.....	4.17	6.65	2.48	1.79	—2.38
30.....	5.21	10.08	4.87	3.91	—1.30
42.....	7.44	11.16	3.72	5.95	—1.49

30° C.

11.....	3.29	4.82	1.53	1.10	—2.19
20.....	7.27	12.02	4.75	5.89	—1.38
30.....	8.45	14.88	6.43	12.50	4.05
42.....	10.42	17.86	7.44	17.36	6.94

38.5° C.

11.....	2.50	4.73	2.23	1.72	—0.78
20.....	6.42	9.67	3.25	11.57	5.15
30.....	10.08	13.89	3.81	16.89	6.81
42.....	11.57	15.24	3.67	19.53	7.96

These experiments are of too limited a nature to justify broad conclusions but certainly indicate that there is little justification for the statement that hot weather is unfavorable for the use of cyanamid while cool weather favors the desirable transformations. Just the reverse was the case in the experiment here reported and the indications are that low temperatures slow up the desirable changes and favor the formation of dicyanodiamid and possibly guanylurea.

#### NITRIFICATION OF CYANAMID AND UREA IN PARTIALLY STERILIZED SOILS

In order to determine the effect of partial sterilization of a soil upon nitrification, varying percentages of phenol were added to 100 gm. samples of soil containing 10 mgm. of nitrogen as cyanamid or urea. The percentages of phenol

are based upon the dry weight of the soil. The analyses, which were made by the colorimetric method, are given in Table VIII and figure 6.

The presence of phenol even in the smallest amounts used decreased nitrification in practically every instance and at percentages above 0.1 per cent prevented nitrification altogether. There was a very slight stimulation of nitrification at 0.01 per cent at the end of two weeks but not later. It is quite evident that phenol is very injurious to the nitrifying organisms, probably to an even greater extent than for other groups of bacteria. A partial sterilization of the soil could not, therefore, prove beneficial to nitrification if the nitrifiers were the first organisms to be killed. In Table VIII it will be observed that in most cases slightly more nitrates are present in the soils containing 1 and 2 per cent of phenol than with 0.2 to 0.4 per cent. This is probably because the larger per-

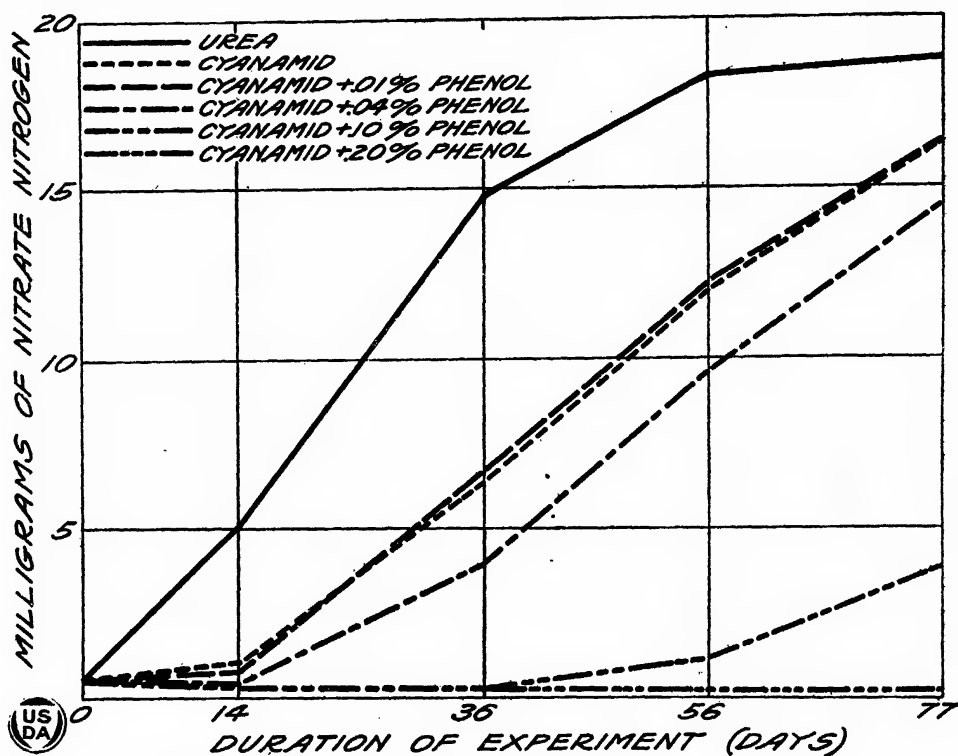


FIG. 6.—Diagram showing the effect of phenol on nitrification

centages of phenol completely sterilized the soil while with the smaller applications some groups of organisms survived and used up the nitrates present. With the highest percentages of phenol the nitrate content of the soil remained at near that of the dry soil prior to incubation. With the lower percentages the phenol practically stopped all nitrification for the first two weeks but there was a partial recovery later.

## PART II. CHANGES WHICH DICYANODIAMID, GUANYLUREA, GUANIDIN, AND BIGUANID NITROGEN UNDERGO OR PRODUCE IN SOIL

### COMPARATIVE RATES OF NITRIFICATION OF DICYANODIAMID, GUANYLUREA SULPHATE, AND AMMONIUM SULPHATE

This experiment was made for the purpose of determining if dicyanodiamid and guanylurea sulphate nitrify in the soil provided a considerable period of time is allowed for the process to take place. For comparison ammonium sulphate

was included. The results, which were obtained by the reduction method, are given in Table IX and figure 7.

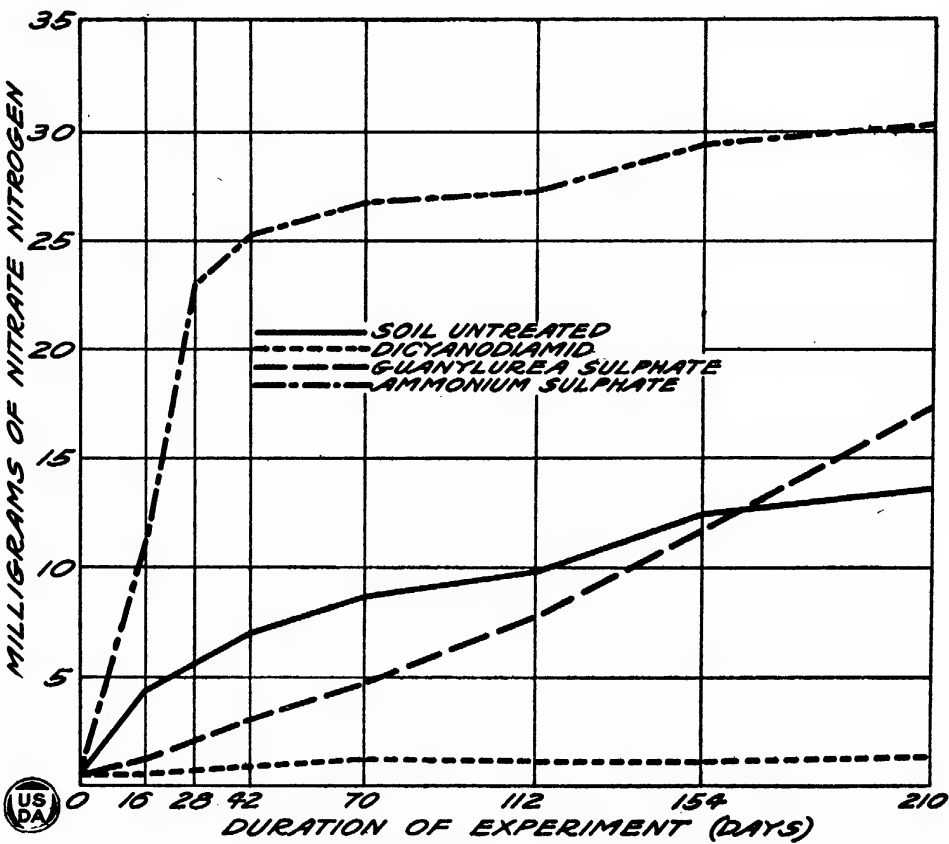


FIG. 7.—Diagram showing the nitrates present in soils receiving dicyanodiamid, guanylurea sulphate, and ammonium sulphate

TABLE VIII.—Nitrate formation from cyanamid in soils containing various percentages of phenol <sup>a</sup>

Fertilizer	Phenol	Nitrates mgm. N	Recovery of N as nitrates mgm. N	Nitrates mgm. N	Recovery of N as nitrates mgm. N
		14 days		36 days	
	Per cent				
Control.....	0	3.13	-----	7.81	-----
Urea.....	0	5.00	1.87	14.88	7.07
Cyanamid.....	0	.81	-2.32	6.79	-1.02
Do.....	0.01	1.00	-2.13	6.38	-1.43
Do.....	.04	.40	-2.73	3.97	-3.84
Do.....	.10	.27	-2.86	.32	-7.49
Do.....	.20	.50	-2.63	.25	-7.56
Do.....	.40	.69	-2.44	.27	-7.54
Do.....	1.00	.30	-2.83	.50	-7.31
Do.....	2.00	.45	-2.68	.74	-7.07
		56 days		77 days	
Control.....	0	8.45	-----	10.78	-----
Urea.....	0	18.38	9.93	18.94	8.16
Cyanamid.....	0	12.50	4.05	16.45	5.67
Do.....	.01	12.02	3.57	16.45	5.67
Do.....	.04	9.62	1.17	14.53	3.75
Do.....	.10	1.18	-7.27	3.83	-6.95
Do.....	.20	.21	-8.24	.25	-10.53
Do.....	.40	.34	-8.11	.28	-10.50
Do.....	1.00	.58	-7.87	.56	-10.22
Do.....	2.00	.78	-7.67	.50	-10.28

<sup>a</sup> Cyanamid and urea were used at the rate of 10 mgm. N per 100 gm. soil.

TABLE IX.—Comparison of the rates of nitrification of dicyanodiamid, guanylurea sulphate, and ammonium sulphate

Treatment (20.9 mgm. N per 100 gm. soil)	16 days		28 days		42 days		70 days		112 days		154 days		210 days	
	Nitrates mgm. N	Increase over control mgm.	Nitrates mgm. N	Increase over control mgm.	Nitrates mgm. N	Increase over control mgm.	Nitrates mgm. N	Increase over control mgm.	Nitrates mgm. N	Increase over control mgm.	Nitrates mgm. N	Increase over control mgm.	Nitrates mgm. N	Increase over control mgm.
Soil, untreated.....	4.31	-----	5.51	-----	6.91	-----	8.65	-----	9.83	-----	12.50	-----	13.75	-----
Dicyanodiamid.....	.45	-3.86	.66	-4.85	.87	-6.04	1.22	-7.43	1.08	-8.75	1.08	-11.42	1.29	-12.46
Guanylurea sul- phate.....	1.17	-3.14	2.12	-3.39	3.03	-3.88	4.65	-4.00	7.73	-2.10	11.72	-.78	17.40	3.65
Ammonium sul- phate.....	10.64	6.33	23.01	17.50	25.26	18.35	26.88	18.23	27.39	17.56	29.65	17.15	30.27	16.52

The retarding effect of dicyanodiamid upon the nitrification processes was very striking. Not only was the dicyanodiamid not converted into nitrates but at the concentration used it almost completely stopped the nitrification of the nitrogen present in the soil organic matter. At the end of 210 days the soil with dicyanodiamid contained 1.29 mgm. of nitrate nitrogen per 100 gm. of air-dried soil as contrasted with 13.75 mgm. in the untreated soil. At the beginning of the experiment the soil contained 0.56 mgm. nitrate nitrogen. If we consider the nitrification of the organic matter in the untreated soil as 100 per cent then the dicyanodiamid retarded the nitrification of the soil nitrogen to the extent of 94.5 per cent.

Guanylurea sulphate likewise not only failed to nitrify readily but partially prevented the nitrification of the soil nitrogen. The injurious effect was greatest at first. After 154 days the nitrate accumulation was nearly as great in the presence of the guanylurea as in the untreated soil. At the end of 210 days this material showed a nitrification of 17.5 per cent. These results would indicate that eventually guanylurea sulphate is transformed in the soil to nitrate nitrogen but the process is so slow that the material can not be considered as a fertilizer. In considering the injurious effects on nitrification noted in these experiments it should be borne in mind that the quantities of guanylurea required to produce the injury were far in excess of any ever likely to be found under field conditions.

#### THE RATE OF DECOMPOSITION OF VARYING QUANTITIES OF DICYANODIAMID

From the work reported above it seemed that dicyanodiamid was not nitrified in the soil even after a considerable period of time. In order to obtain further data on this point experiments were made using smaller quantities of the material and both ammonia and nitrate determinations were made at intervals. In analyzing the samples 25 gm. were taken for each ammonia determination while the remainder of the sample was extracted with 500 cc. of water, filtered and the dicyanodiamid nitrogen determined in 50 cc. aliquots, nitrates being determined by the reduction method in 100 cc. aliquots. The method for dicyanodiamid was tested prior to starting the experiments by analyzing several soil samples containing different known quantities of dicyanodiamid. The average recovery was 98.12 per cent with extreme limits of 95.93 and 99.34 per cent. The data, given in Table X, and shown diagrammatically in figure 8, represent the averages of duplicate determinations in the case of ammonia and nitrate nitrogen and the averages of triplicates for dicyanodiamid. The reduction method was used for the nitrate determinations.

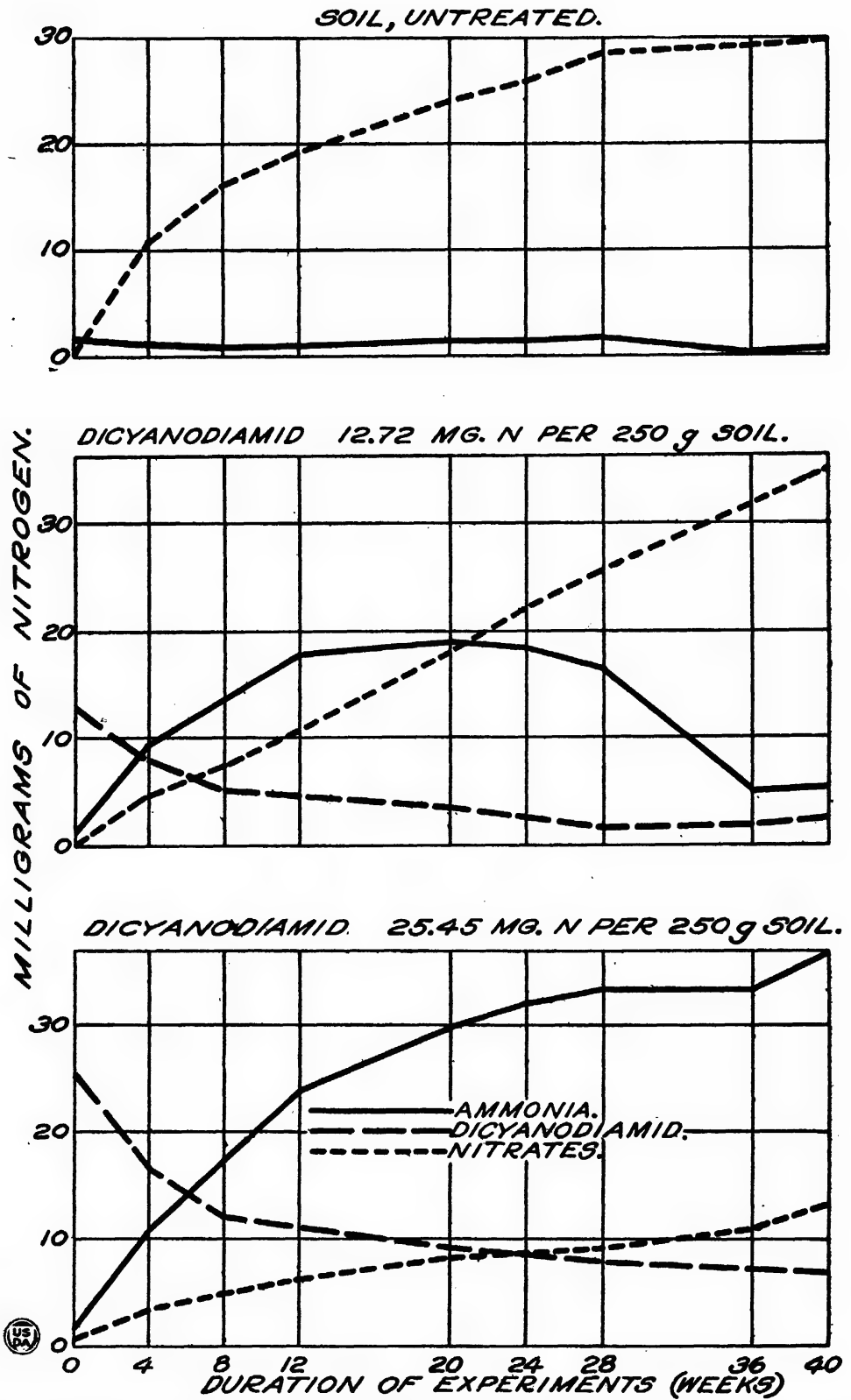


Fig. 8.—Diagram showing the rate of formation of ammonia and nitrates in soils receiving dicyanodiamid

The disappearance of dicyanodiamid from the soil, as shown in Table X, takes place fairly rapidly. At the two concentrations used only 61 to 65 per cent of the dicyanodiamid was present after four weeks. With further incubation of the mixtures the dicyanodiamid decreased slowly, but 18 to 27 per cent remained even after 40 weeks. It is quite probable, however, that all of the analytic figures for dicyanodiamid are too high since silver nitrate precipitates several other decomposition products of cyanamid. These other products, if present at all, were doubtless in small amounts. The figures show that dicyanodiamid slowly decomposes in the soil to ammonia which is in turn more slowly converted into nitrates. With 12.72 mgm. of dicyanodiamid nitrogen per 250 gm. of soil actual nitrification of the added nitrogen apparently did not commence until after 36 weeks. With twice this quantity of dicyanodiamid the nitrate present after 40 weeks was still less than in the untreated soil.

Dicyanodiamid inhibits the action of the nitrifying organisms of the soil causing an accumulation of ammonia in the soil during the early stages of its decomposition. Normal nitrification is not resumed until the concentration of undecomposed dicyanodiamid is reduced to such a point that it is no longer markedly toxic to the soil organisms. The ammonia accumulation in soils containing dicyanodiamid is due both to the decomposition of the organic matter present and also to the breaking up of the dicyanodiamid itself.

The recovery of nitrogen from the soils receiving the largest application of dicyanodiamid was never higher than 85 per cent during the first 36 weeks. This indicates that either the dicyanodiamid was absorbed by the soil to a certain extent and was not extracted with water or else some of it was converted into compounds that were not determined. In this experiment at the end of 40 weeks, apparently all of the added nitrogen was present as nitrate, ammonia and dicyanodiamid. With the smaller application the recovery of nitrogen was more than 100 per cent in some instances. This may have been due to a slight stimulation of the ammonification of the soil organic matter by the dicyanodiamid but more likely was experimental error. Extreme accuracy can not be expected with the methods used, especially when dealing with such small amounts of nitrogen in soils containing various forms of nitrogen.

#### THE EFFECT OF DICYANODIAMID UPON THE RATE OF NITRIFICATION OF AMMONIUM SULPHATE

Since previous experiments had shown that soils receiving dicyanodiamid contain only a small fraction of the quantity of nitrates present in untreated soil for several days or weeks following the application, it was of interest to know to what extent the dicyanodiamid prevents the nitrification of ammonia nitrogen. The results of the analyses by the reduction method are given in Table XI and shown diagrammatically in figure 9.

The procedure adopted was, in general, the same as used previously except that ammonia determinations were not made. Two samples of soil taken from the same field but at different times were used. The results reported in the upper portion of the table, using the two smaller rates of application of dicyanodiamid, were with one soil sample while those with the two higher rates were with the other sample. This slight variation had little effect upon the results.

The marked toxicity of very small percentages of dicyanodiamid is very strikingly brought out in Table XI. Even as little as 0.1 mgm. per 100 gm. of soil delayed the nitrification of ammonium sulphate. As the concentration of dicyanodiamid was increased this effect became more pronounced until at a concentration of 10.5 mgm. of dicyanodiamid per 100 gm. of soil absolutely no nitrification of ammonia nitrogen occurred in 210 days and at the end of 280 days a conversion of only 26.12 per cent of the added ammonia nitrogen had taken place

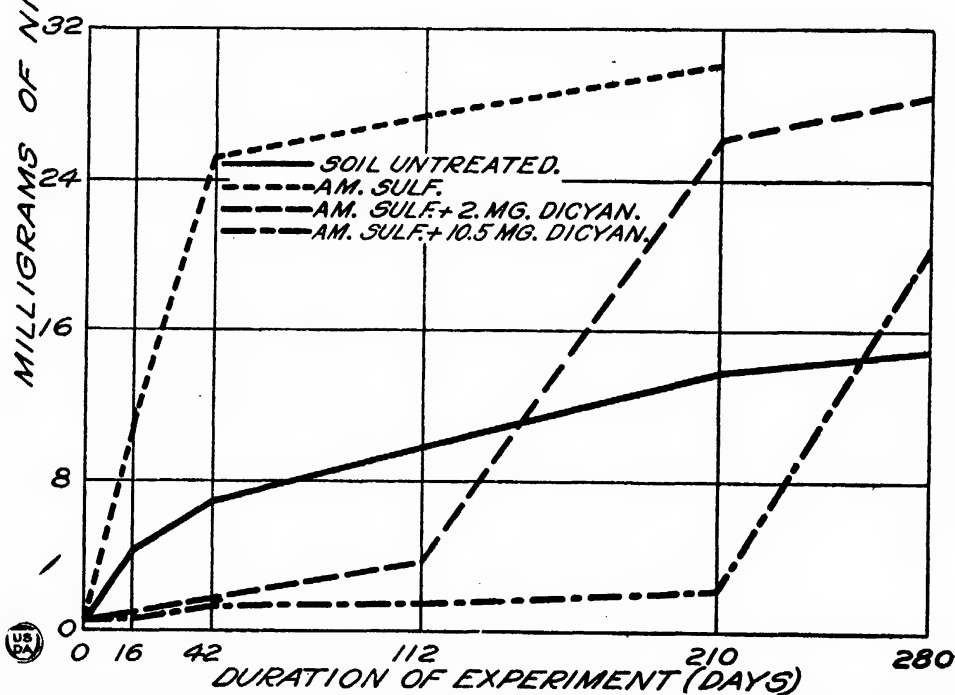
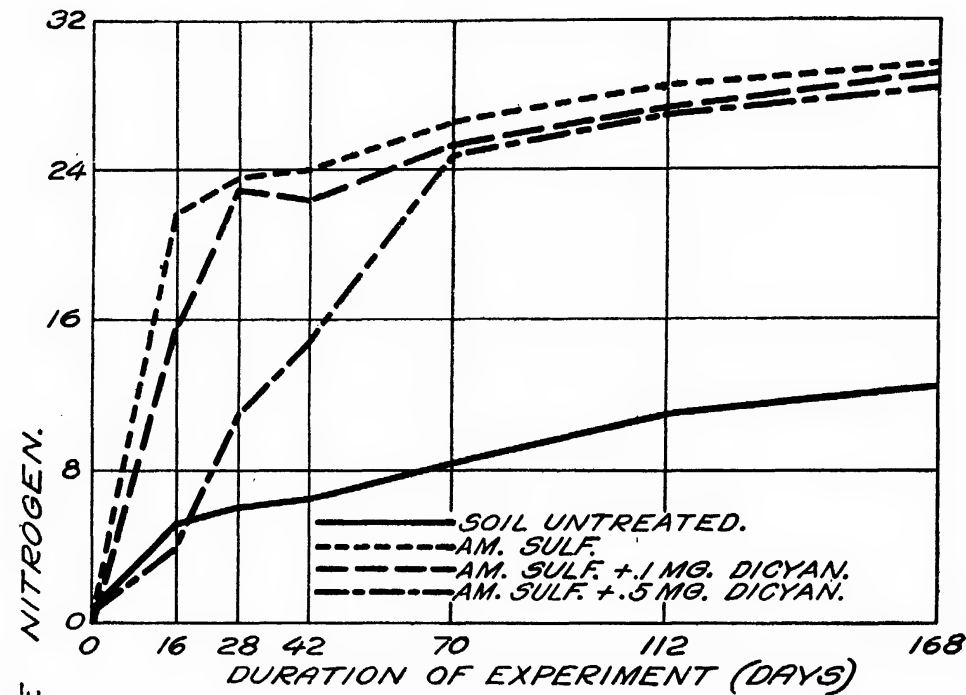


FIG. 9.—Diagram showing the effect of dicyanodiamid upon the nitrification of ammonium sulphate

TABLE X.—Ammonification and nitrification of varying quantities of dicyanodiamid<sup>a</sup>

Incubation period	Soil alone 250 gm		Dicyanodiamid 12.72 mgm. N per 250 gm. soil					Dicyanodiamid 25.45 mgm. N per 250 gm. soil				
	Am-mo-nia mgm. N	Ni-trates mgm. N	Dicy-ano-dia-mid mgm. N	Am-mo-nia mgm. N	Ni-trates mgm. N	Recovery of N		Dicy-ano-dia-mid mgm. N	Am-mo-nia mgm. N	Ni-trates mgm. N	Recovery of N	
						Am-mo-nia and ni-trates mgm. N	Total, in-cluding dicy-ano-dia-mid mgm. N				Am-mo-nia and ni-trates mgm. N	Total, in-cluding dicy-ano-dia-mid mgm. N
<i>Weeks</i>												
4.....	0.43	10.61	7.77	9.38	4.56	2.90	10.67	16.65	10.47	3.25	2.68	19.33
8 <sup>b</sup> .....		16.16	5.20		7.20			12.18		4.73		
12.....	.83	19.14	4.73	17.63	10.48	8.14	12.87	11.04	23.72	5.99	9.74	20.78
20.....	1.35	23.91	3.54	18.96	18.02	11.72	15.26	9.30	29.18	8.11	12.03	21.33
24.....	1.30	25.91	2.40	18.26	22.14	13.19	15.59	7.58	31.80	8.38	12.97	20.58
28.....	1.52	28.43	1.36	16.41	25.50	11.96	13.32	7.80	33.21	8.92	12.18	19.98
36.....	.00	29.26	1.86	5.04	31.51	7.29	9.15	7.24	33.16	10.45	14.35	21.59
40.....	.39	30.07	2.51	5.22	34.98	9.74	12.25	6.83	36.58	12.80	18.92	25.76

<sup>a</sup> At the beginning of the experiment 250 gm. of the soil contained 1.88 mgm. of nitrogen as ammonia and 1.63 mgm. as nitrate.

<sup>b</sup> The ammonia determinations were lost at this period of analysis.

TABLE XI.—The effect of dicyanodiamid upon the rate of nitrification of ammonium sulphate<sup>a</sup>

Incubation period	Soil alone 100 gm. nitrates mgm. N	Ammonium sulphate (20.9 mgm. N)			Dicyanodiamid 0.1 mgm. (0.066 mgm. N) and ammonium sulphate			Dicyanodiamid 0.5 mgm. (0.331 mgm. N) and ammonium sulphate		
		Ni-trates mgm. N	Recov-ery as ni-trates mgm. N	Con-ver-sion	Ni-trates mgm. N	Recov-ery as ni-trates mgm. N	Con-ver-sion (per cent)	Ni-trates mgm. N	Recov-ery as ni-trates mgm. N	Con-ver-sion (per cent)
<i>Days</i>				<i>Per cent</i>						
0.....	0.65	0.65			0.65			0.65		
16.....	4.98	21.64	16.66	79.71	14.63	9.65	46.17	4.18	-0.80	0.00
28.....	6.01	23.48	17.47	83.59	22.82	16.81	80.43	10.66	4.65	22.25
42.....	6.45	24.05	17.60	84.21	22.38	15.93	76.22	14.53	8.08	38.60
70.....	8.24	26.64	18.40	88.04	25.39	17.15	82.06	24.89	16.65	79.66
112.....	11.03	28.50	17.47	83.59	27.22	16.19	77.46	26.99	15.96	76.36
168.....	12.34	29.83	17.49	83.68	29.35	17.01	81.39	28.69	16.35	78.23
		Ammonium sulphate (20.9 mgm. N)			Dicyanodiamid 2 mgm. (1.33 mgm. N) and ammonium sulphate			Dicyanodiamid 10.5 mgm. (6.96 mgm. N) and ammonium sulphate		
16.....	4.31	10.64	6.33	30.28	1.05	-3.26	0.00	0.94	-3.37	0.00
42.....	6.91	25.26	18.35	87.80	1.73	-5.18	.00	1.25	-5.66	.00
112.....	9.83	27.39	17.56	84.02	3.94	-5.89	.00	1.44	-8.39	.00
210.....	13.75	30.27	16.52	79.04	26.41	12.66	60.57	2.36	-11.39	.00
280.....	15.00				28.58	13.58	64.97	20.46	5.46	26.12

<sup>a</sup> Ammonium sulphate was used in all cases at the rate of 20.9 mgm. N per 100 gm. of soil.



## THE EFFECT OF DICYANODIAMID UPON THE RATE OF AMMONIFICATION OF UREA

These experiments were planned to determine if dicyanodiamid prevents or retards ammonification, as was found to be true in the case of nitrification. The dicyanodiamid and urea were added in solution to 250 gm. samples of soil. No calcium carbonate was used. The results of the ammonia determinations are given in Table XII and shown in part in figure 10.

From the data it will be seen that concentrations of dicyanodiamid as high as 315.2 mgm. per 250 gm. of soil had no appreciable effect upon the rate of ammonification of urea. The recovery of more than 100 per cent of the added

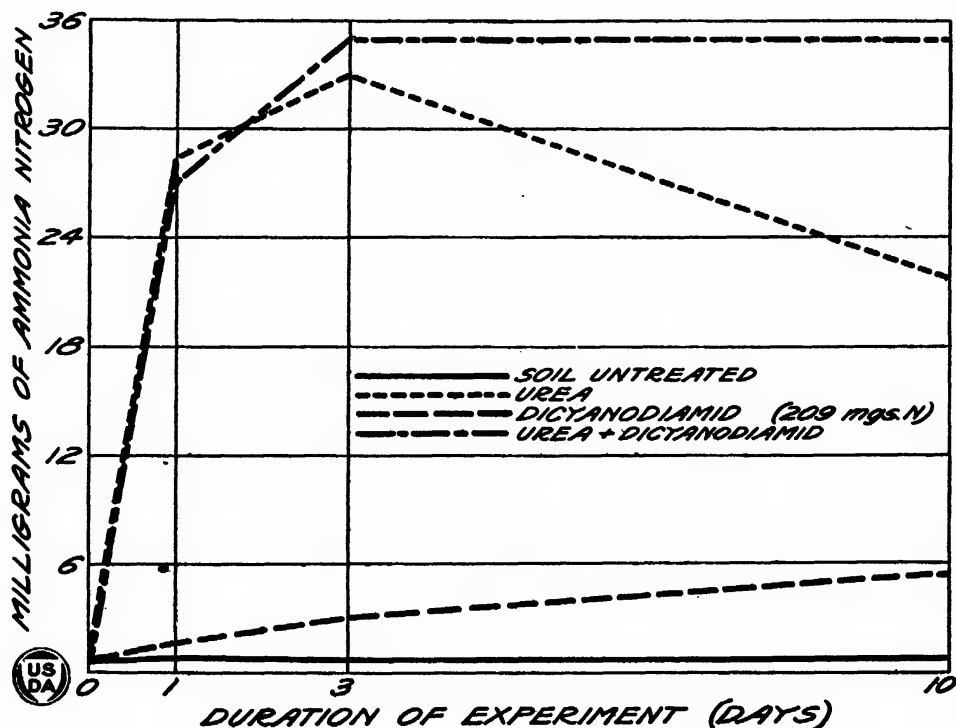


FIG. 10.—Diagram showing the effect of dicyanodiamid upon the ammonification of urea

urea nitrogen in most of the analyses indicates that the added nitrogenous compounds accelerated the ammonification of the organic soil nitrogen. Another possible explanation is the fact that soluble salts tend to accumulate at the surface of a soil layer due to evaporation, and it is quite possible that in obtaining the 25 gm. of soil from the 250 gm. samples the mixture was not as thoroughly mixed as it should have been. This would mean that too large a portion of the surface soil was included in the samples analyzed. Regardless of the discrepancy, it is quite evident that dicyanodiamid is not especially toxic, if at all, to the ammonifying organisms. In a fertile soil containing appreciable dicyanodiamid we would therefore expect to find an ammonia accumulation and practically no nitrates.

TABLE XII.—*The effect of dicyanodiamid upon the ammonification of urea*

Treatment (mgm. N per 250 gm. soil)	1 day		3 days		10 days	
	Ammonia mgm. N	Urea as am- monia mgm. N	Ammonia mgm. N	Urea as am- monia mgm. N	Ammonia mgm. N	Urea as am- monia mgm. N
Soil alone.....	0.83	-----	0.65	-----	0.54	-----
Urea 26.15.....	28.43	-----	32.98	32.33	22.37	<sup>a</sup> 21.83
Dicyanodiamid 13.06.....	1.40	-----	2.10	-----	2.85	-----
Dicyanodiamid 26.12.....	1.88	-----	2.63	-----	3.33	-----
Dicyanodiamid 52.24.....	2.10	-----	3.95	-----	3.81	-----
Dicyanodiamid 104.50.....	1.88	-----	3.73	-----	3.77	-----
Dicyanodiamid 156.75.....	1.66	-----	2.45	-----	3.38	-----
Dicyanodiamid 209.....	1.62	-----	2.98	-----	3.33	-----
Dicyanodiamid 13.06 and urea 26.15.....	28.25	26.85	34.65	32.55	38.07	35.22
Dicyanodiamid 26.12 and urea 26.15.....	28.64	26.76	34.70	32.07	38.77	35.44
Dicyanodiamid 52.24 and urea 26.15.....	27.81	25.71	36.14	32.19	37.28	33.47
Dicyanodiamid 104.50 and urea 26.15.....	27.59	25.71	36.49	32.76	39.17	35.40
Dicyanodiamid 156.75 and urea 26.15.....	26.06	24.40	33.91	31.46	34.34	30.96
Dicyanodiamid 209 and urea 26.15.....	27.15	25.53	34.96	31.98	35.09	31.76

<sup>a</sup> A nitrate determination made on this sample showed 14.26 mgm. nitrate nitrogen. This rapid conversion of the urea into nitrates accounts for the low percentage of ammonia at this period.

#### THE EFFECT OF GUANYLUREA SULPHATE UPON THE RATE OF AMMONIFICATION AND NITRIFICATION OF UREA

The figures reported in Table IX showed that guanylurea sulphate retarded nitrification but to a lesser extent than did dicyanodiamid. In order to obtain further data upon this material a more extensive set of experiments was carried out to determine the effect of guanylurea sulphate upon both ammonification and nitrification. The guanylurea sulphate used was very carefully prepared and extracted with acetone until no trace of dicyanodiamid could be detected. Both the urea and guanylurea were added in solution to 250 gm. samples of soil containing no calcium carbonate.

Nitrates and ammonia were determined on the same sample, the reduction method being used for the nitrate determinations. In the mixtures containing guanylurea sulphate this compound was removed by precipitation with silver sulphate and potassium hydroxid before analyzing for nitrates. The data are given in Table XIII. Figure 11 was based upon these results.

It will be seen that guanylurea sulphate, in the concentrations used in the above experiments, had very little if any effect upon the rate of conversion of urea to ammonia in the soil but it did markedly slow down the nitrification of this material. The higher the concentration of guanylurea sulphate the greater was the inhibiting effect but it was not nearly as great as in the case of dicyanodiamid. Where guanylurea sulphate was applied singly there was no ammonia accumulation as in the case of dicyanodiamid (Table X) but the material did slowly break up into ammonia. The small quantity formed was converted into nitrates almost immediately. Comparing the results of Table XIII with those of Table X it will be seen that while dicyanodiamid is converted into ammonia fairly rapidly a very small amount of the material will prevent the oxidation of the ammonia to nitrates. On the other hand, guanylurea ammonifies only very slowly but it is much less toxic to the nitrifying organisms. From these results we would conclude that both of the compounds, particularly dicyanodiamid, are undesirable in soils. However, it should be emphasized that since such exceedingly large percentages of guanylurea sulphate are required to appreciably slow up nitrification there is little probability that injury would be observed under practical conditions.

# NITRIFICATION OF GUANIDIN NITRATE, NITRO GUANIDIN, BIGUANID NITRATE AND UREA

In continuation of the study of the transformation products of cyanamid a series was started using guanidin nitrate, nitro guanidin and biguanid nitrate with urea for comparison. The undesirability of using nitrate salts of the organic radicals was appreciated but at the time of starting the experiments only these salts were available. Adequate controls were arranged where the soil received

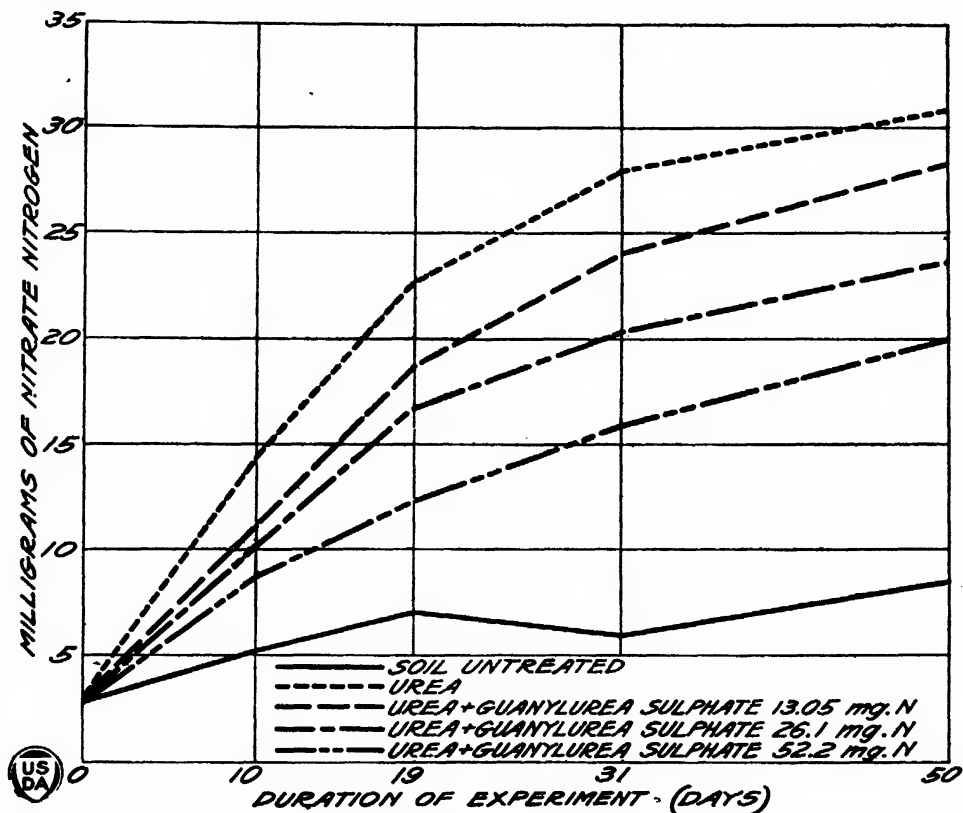


FIG. 11.—Diagram showing the effect of guanylurea sulphate upon the rate of nitrification of urea

sodium nitrate equivalent to the nitrate nitrogen in the compounds being studied. The rate of application for all of the salts was 10 mgm. nitrogen other than nitrate per 100 gm. soil. The colorimetric method was used for nitrate determinations. The data which are the averages of duplicate determinations are given in Table XIV.

TABLE XIII.—*Ammonification and nitrification of urea in the presence of guanyl-urea sulphate*

SOIL ALONE (250 GM.)

Time  <i>Days</i>	Ammonia mgm. N	Nitrates mgm. N	Recovery of Nitrogen <sup>a</sup>		
			As ammonia mgm. N	As nitrates mgm. N	Total mgm. N
0.....	1.31	3.07	-----	-----	-----
1.....	.83	-----	-----	-----	-----
10.....	.54	5.08	-----	-----	-----
19.....	.17	7.04	-----	-----	-----
31.....	.18	5.92	-----	-----	-----
50.....	.00	8.50	-----	-----	-----
100.....	.31	9.66	-----	-----	-----

UREA 26.15 MGM. N

1.....	28.43	-----	27.60	-----	-----
10.....	22.37	14.26	21.83	9.18	31.01
19.....	10.00	22.65	9.83	15.61	25.44
31.....	3.86	27.88	3.68	21.96	25.64
50.....	1.88	30.88	1.88	22.38	24.26
100.....	1.93	34.37	1.62	24.71	26.33

GUANYLUREA SULPHATE 13.05 MGM. N

1.....	1.53	-----	.70	-----	-----
10.....	.41	5.92	-.13	.84	.71
19.....	.20	7.83	.03	.79	.82
31.....	.45	8.71	.27	2.79	3.06
50.....	.54	10.84	.54	2.34	2.88

GUANYLUREA SULPHATE 26.1 MGM. N

1.....	1.40	-----	.57	-----	-----
10.....	.76	6.36	.22	1.28	1.50
19.....	.18	7.64	.01	.60	.61
31.....	.45	8.29	.27	2.37	2.64
50.....	.48	10.29	.48	1.79	2.27

GUANYLUREA SULPHATE 52.2 MGM. N

1.....	1.62	-----	.79	-----	-----
10.....	3.16	6.09	2.62	1.01	3.63
19.....	1.66	8.71	1.49	1.67	3.16
31.....	1.75	8.21	1.57	2.29	3.86
50.....	.65	9.02	.65	.52	1.17

GUANYLUREA SULPHATE 13.05 MGM. N—UREA 26.15 MGM.

1.....	26.49	-----	24.96	-----	-----
10.....	28.69	11.06	28.28	5.14	33.42
19.....	22.15	18.60	21.95	10.77	32.72
31.....	19.43	24.04	18.98	15.33	34.31
50.....	12.98	28.32	12.44	17.48	29.92

GUANYLUREA SULPHATE 26.1 MGM. N—UREA 26.15 MGM. N

1.....	26.75	-----	25.35	-----	-----
10.....	30.44	10.21	29.68	3.85	33.53
19.....	27.68	16.75	27.50	9.11	36.61
31.....	25.57	20.17	25.12	11.88	37.00
50.....	20.61	23.54	20.13	13.25	33.38

GUANYLUREA SULPHATE 52.2 MGM. N—UREA 26.15 MGM. N

1.....	25.70	-----	24.08	-----	-----
10.....	31.20	8.71	28.04	2.62	30.66
19.....	27.88	12.32	26.22	3.61	29.83
31.....	30.13	15.93	28.38	7.72	36.10
50.....	27.58	20.07	26.93	11.05	37.98

<sup>a</sup> The figures for the recovery of nitrogen apply to urea only, where guanylurea sulphate and urea were used together

TABLE XIV.—*Comparison of the rates of nitrification of guanidin nitrate, nitro guanidin, biguanid nitrate and urea*

Treatment	Mgm. N added other than nitrates	14 days		29 days		50 days		71 days	
		Ni-trates mgm. N	In-crease over control mgm.	Ni-trates mgm. N	In-crease over control mgm.	Ni-trates mgm. N	In-crease over control mgm.	Ni-trates mgm. N	In-crease over control mgm.
Soil alone.....	0	3.85	-----	5.00	-----	6.94	-----	7.91	-----
Urea.....	10	4.93	1.08	10.78	5.78	14.20	7.26	17.36	9.45
Guanidin nitrate.....	10	5.65	— .60	8.33	.52	11.61	1.62	12.50	3.31
Sodium nitrate <sup>a</sup> .....	0	6.25	-----	7.81	-----	9.99	-----	9.19	-----
Nitro guanidin.....	10	4.71	.86	6.10	1.10	8.68	1.74	8.80	.89
Biguanid nitrate.....	10	4.98	— .32	7.14	— .21	9.19	.97	8.91	.90
Sodium nitrate <sup>b</sup> .....	0	5.30	-----	7.35	-----	8.22	-----	8.01	-----

<sup>a</sup> The application of sodium nitrate was equivalent to the nitrate present in the guanidin nitrate treatment.

<sup>b</sup> The application of sodium nitrate was equivalent to the nitrate present in the biguanid nitrate treatment.

It will be observed that guanidin nitrate slightly inhibited nitrification during the first two weeks but later there was a gradual increase in nitrate nitrogen until at the end of the experiment (71 days) 33 per cent of the guanidin nitrogen had been converted into the nitrate form. The behavior of this compound was, therefore, quite similar to that already noted in the case of guanylurea sulphate. Nitro guanidin did not have an injurious effect on nitrate formation but nevertheless it did not nitrify to any marked extent. The maximum nitrification which occurred at the end of 50 days was 17.4 per cent. Since one-fourth of the nitrogen present in nitro guanidin is in the nitrite form it is possible that the nitrate formation was wholly from the nitrite radical.

Biguanid nitrate produced a very slight decrease in nitrates during the first month but the decrease was practically within experimental error. At the end of 50 days 9.7 per cent of the added biguanid nitrogen was recovered as nitrates and this percentage was not increased by longer incubation.

Urea, which was included for comparison, nitrified gradually until at the end of the experiment the maximum nitrification of 94.5 per cent was reached.

#### NITRIFICATION OF GUANIDIN CARBONATE AND AMMONIUM SULPHATE

Subsequent to starting the experiment discussed above a sample of guanidin carbonate was obtained and a new series of experiments started similar to the first using this material in comparison with ammonium sulphate. Three rates of application were used namely, 5, 10 and 20 mgm. N per 100 gm. soil. The figures given in Table XV are the average of duplicate determinations obtained by the colorimetric method. These results are also shown in figure 12.

TABLE XV.—*Comparison of the rates of nitrification of guanidin carbonate and ammonium sulphate*

Treatment	Mgm. N added	19 days		35 days		56 days		75 days	
		Ni-trates mgm. N	In-crease over control mgm.	Ni-trates mgm. N	In-crease over control mgm.	Ni-trates mgm. N	In-crease over control mgm.	Ni-trates mgm. N	In-crease over control mgm.
Soil alone.....	0	6.86	-----	7.66	-----	8.99	-----	8.54	-----
Ammonium sulphate.....	5	10.21	3.35	11.58	3.92	14.45	5.49	13.72	5.18
Do.....	10	10.00	3.14	16.95	9.29	18.95	9.96	18.95	10.41
Do.....	20	9.44	2.58	26.39	18.73	23.18	19.19	27.79	19.25
Guanidin carbonate.....	5	4.68	— 2.18	10.22	2.56	12.57	3.58	12.71	4.17
Do.....	10	2.07	— 4.79	9.31	1.65	15.43	6.44	17.66	9.12
Do.....	20	1.38	— 5.48	1.71	— 5.95	9.74	.75	18.28	9.74

It will be observed that guanidin carbonate very markedly inhibited nitrification during the first three to five weeks, depending upon the rate of application. Later the nitrification was quite rapid and nearly complete. At the last period of analysis the percentage nitrification was 83.4, 91.2, and 48.7 for the 5, 10, and 20 mgm. of nitrogen added. Undoubtedly, if the experiment had been continued

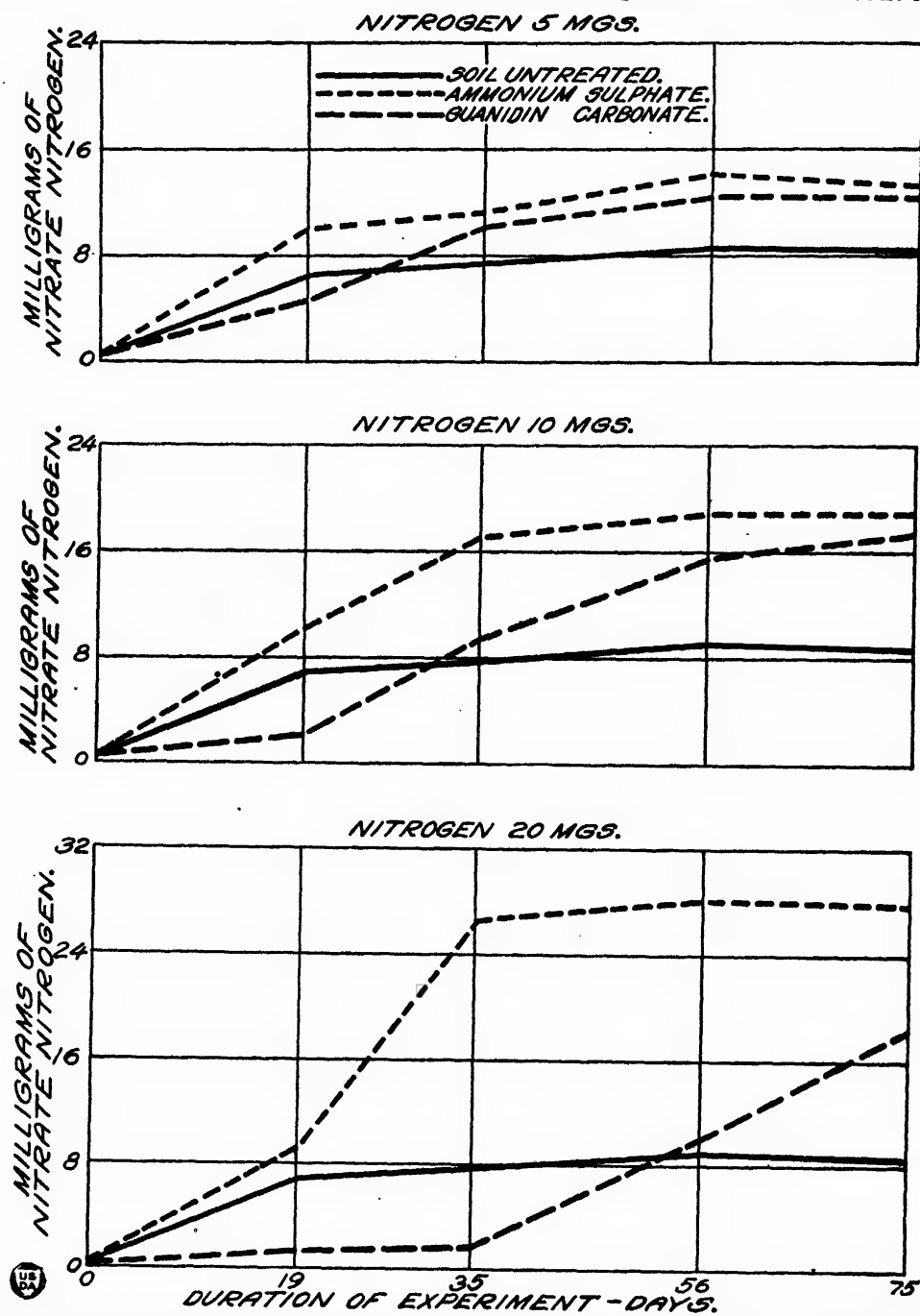


FIG. 12.—Diagram showing the comparative rates of nitrification of varying amounts of guanidin carbonate and ammonium sulphate

longer the largest rate of application would have shown a percentage nitrification comparable with the other rates.

The nitrification of ammonium sulphate took place fairly rapidly and was nearly complete at the end of the experiment. In fact, with the smaller rates of application the figures show slightly more than 100 per cent nitrification. This was probably due to a slight stimulation of nitrification of the soil organic matter.

## SUMMARY

The study of the changes which cyanamid nitrogen and its transformation products undergo in the soil emphasized the following points:

1. Cyanamid was rapidly converted into its various decomposition products, chiefly urea and ammonia. Tests for cyanamid showed none present after 5 to 10 days depending upon the rate of application. Urea was likewise present for only very short periods, being broken up into ammonia so rapidly that no accumulation occurred. Other decomposition products of cyanamid which were precipitated by silver nitrate were present in soils receiving cyanamid for a considerable period after applying. No attempt was made to isolate these compounds but they probably consisted largely of dicyanodiamid and possibly guanlyurea.

2. The nitrification of cyanamid usually took place more slowly than that of urea or ammonium sulphate and the larger the application the slower the process. After the initial retarding period of two to four weeks nitrification proceeded at a more nearly normal rate. Some of the transformation products of cyanamid are toxic to the nitrifying bacteria and where present in appreciable amounts indirectly cause an ammonia accumulation.

3. Cyanamid which had been hydrated and oiled nitrified at practically the same rate as did the untreated material. The slight difference in favor of the latter is thought to have been due to the presence of a trace of dicyanodiamid in the hydrated material and not to the presence of the oil.

4. The addition of calcium carbonate to soil receiving cyanamid retarded nitrification, contrary to results obtained with urea and ammonium sulphate. This peculiar behavior was probably due to an increase in dicyanodiamid formation in the presence of the calcium carbonate.

5. The rate of nitrification of cyanamid at different moisture contents was the highest at one-fourth of saturation (10 per cent) while for urea the rate was higher with both one-half and three-fourths than with one-fourth. The maximum nitrate accumulation for both fertilizers was with 30 per cent moisture but in the case of cyanamid the nitrates present after two months was very nearly the same with either 10, 21 or 30 per cent. With 40 per cent moisture there was little if any nitrate formation.

6. Nitrate formation from cyanamid was more rapid and complete at 38.5° C. than at lower temperatures. At room temperature no nitrification had taken place in one experiment after 42 days. At 30° C. the results were intermediate. Under similar conditions urea nitrified at all temperatures, the rate rapidly increasing up to 30° C. but decreasing at 38.5° C.

7. The partial sterilization of soil with phenol practically stopped all nitrification of cyanamid.

8. Dicyanodiamid when added to soil slowly disappeared, more than one-half being decomposed during a period of two months. The nitrogen accumulated in the soil as ammonia which was not readily nitrified. With 12.72 mgm. of nitrogen as dicyanodiamid per 250 gm. of soil 36 weeks were required for any of the added nitrogen to nitrify while with larger applications no nitrification took place in 40 weeks. In all cases where dicyanodiamid was applied the nitrate formation from the soil organic matter was markedly retarded.

9. The nitrification of ammonium sulphate in the presence of dicyanodiamid was prevented for a period of 210 days where the dicyanodiamid was used at the rate of 10.5 mgm. per 100 gm. soil. Even as little as 0.1 mgm. per 100 gm. of soil greatly delayed nitrification.

10. The rate of ammonification of urea was not appreciably affected by concentrations of dicyanodiamid as high as 315.2 mgm. per 250 gm. soil.

11. Guanylurea sulphate decomposed to ammonia very slowly and the ammonia so formed did not accumulate but was nitrified. Where urea was used with guanylurea sulphate the latter did not affect ammonification but did inhibit nitrification for some weeks. The injurious effect was not nearly as great as in the case of dicyanodiamid and would probably not be observed at all under field conditions.

12. Salts of guanidin, including guanidin nitrate and guanidin carbonate depressed nitrification for several weeks, the period depending upon the rate of application. Thereafter nitrates formed quite rapidly and after 75 days guanidin carbonate had nitrified to the extent of 83, 91 and 49 per cent for the three rates of application used. Nitro guanidin showed a maximum nitrification of 17 per cent after 50 days.

13. Biguanid nitrate acted practically like an inert material. A 3 per cent depression in nitrates at first was followed by a 9 per cent nitrification after 50 days. These slight differences are nearly within experimental error.

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# GROSSULARIA ECHINELLA, A SPINY-FRUITED GOOSEBERRY FROM FLORIDA<sup>1</sup>

By FREDERICK V. COVILLE

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While in Tallahassee, Fla., early this spring, the writer was invited by Dr. Roland M. Harper, of the State geological survey, and Dr. H. Kurz, of the State College for Women, to join them in a visit to Lake Miccosukee where a few weeks before they had found a wild gooseberry, at that time not yet in flower. The plant promised to be of great interest, for although the name "gooseberry" is often misapplied in the southeastern United States to the fruit of the various species of deerberry, or Polycodium, no true gooseberry had ever been reported from Florida.

On March 2, 1924, we made the 26-mile drive from Tallahassee to Dogwood Landing, on the east shore of the northwest arm of Lake Miccosukee, about a mile east of the main buildings of the Norias Club. A few hundred yards east of the landing, in the strip of woodland bordering the lake, we came upon the first plant, in full leaf and flower. It had been our expectation that it would prove to be the Georgia gooseberry, *Grossularia curvata*, a rare southern species, the type locality of which is on the slopes of Stone Mountain, Ga. The plant found, however, was recognized immediately as not *Grossularia curvata* but a species new to science, differing conspicuously from *curvata* in the coarse bristly gland-tipped hairs of the ovary, which develops into an exceedingly spiny fruit. This characteristic has suggested the species name *echinella*, which indicates the resemblance of the berry to a little hedgehog.

On March 27 Doctor Kurz and the writer again visited the locality. Unusually cold weather had prevailed and many of the gooseberry bushes were still in full flower. Some of the young green spiny fruits had already reached a diameter of five-eighths of an inch. On April 7 Doctor Kurz visited the place again and sent the writer fresh pollen from newly expanded flowers, and half mature fruits, the largest seven-eighths of an inch in diameter.

## TECHNICAL DESCRIPTION

***Grossularia echinella*, sp. nov.**

Plant a shrub 0.5 to 1 meter in height, often forming patches several meters in diameter, the branches spreading and recurved, sometimes rooting at the tip; stems with spines at the nodes but devoid of bristles except occasionally on vigorous basal shoots; spines single, double, or triple, stout, dark reddish brown, sometimes turning gray with age, reaching a length of 1.5 cm.; outer bark of 1-year-old branches dull white to almost buff, splitting with the growth of the twig and exposing the inner dark reddish brown bark; petioles pubescent, usually a little longer than the leafblades but sometimes exceeded by them and usually bearing near the base a few large gland-tipped and often plumose hairs;

<sup>1</sup> Received for publication, Apr. 1, 1924.

leafblades 1 to 2 cm. long, sometimes smaller, on vigorous shoots of the season, longer, on strong basal sprouts sometimes reaching 3 cm., orbicular in outline, truncate to rounded at the base, 3-lobed, each lobe with a few rounded teeth, sparingly pubescent or nearly glabrous on both surfaces; peduncles 5 to 12 mm. long, pubescent and usually with scattered longer gland-tipped hairs, one-flowered or occasionally two-flowered; bracts usually 2, pubescent, entire; pedicels 1 to 5 mm. long, densely pubescent and often with scattered gland-tipped hairs like those of the ovary; flower 15 to 20 mm. in length, green to greenish white; ovary densely clothed with stout bristly hairs tipped with cup-shaped glands; calyx tube green, pubescent on the outside, sparingly pubescent on the inside toward the base, cylindrical, 3 to 4 mm. long, the lobes green to greenish white, linear, pubescent on the outside, glabrous on the inside, reflexed, 4 to 6 mm. long; petals about 2 mm. long, greenish white to green, each inrolled into a truncate tube with a spreading or revolute rim at the summit; stamens extending nearly 1 cm. beyond the petals; filaments greenish white to green, usually sparingly hairy; anthers purple, about 1.5 mm. long when dry, sometimes 2.5 mm. long when fresh, usually with a line of long white hairs (or occasionally a green and gland-tipped hair) on the back between the anther sacs; styles green, a little longer than the stamens, very sparingly hairy within, as well as beyond, the calyx tube; largest berry, not yet mature, 22 mm. long, 22 mm. broad, including the prickles, the body 16 mm. in length, 12 mm. in thickness, translucent, uncolored;<sup>2</sup> fruit prickles very numerous, vivid green, somewhat flattened, longer toward the middle of the berry than at the ends, reaching a length of 5 mm., the larger nearly 1 mm. wide at the base, each ending in a green cup-shaped gland. (See Pl. 1.)

Type specimen No. 1,112,807, United States National Herbarium, collected March 2, 1924, a few hundred yards south of Dogwood Landing, east shore of the northwest arm of Lake Miccosukee, Jefferson County, Florida, by Frederick V. Coville.

In the somber surroundings of gray tree trunks, gray Spanish-moss (*Dropogon usneoides*), and gray-brown leaf litter, the patches of this plant were conspicuous for the brilliant green of their new foliage. The only other shrub conspicuously green at the time the gooseberry came into bloom was red buckeye, *Aesculus pavia*, which also was in full leaf and nearly ready to flower. Although its stems are viciously spiny, the new *Grossularia* when in bloom is of graceful and pleasing appearance, the slender, elegant, pale green flowers hanging down singly from the broadly arching branches like aquamarines pendent from an emerald necklace.

The new species differs from its nearest relative, *Grossularia curvata*, in the white or whitish color of the 1-year-old twigs; the ovary densely clothed with stout gland-tipped hairs; the cylindrical, pubescent calyx tube 3 to 4 mm. in length and longer than broad; the green or greenish color of the calyx lobes; the inrolled tubular character of the petals; and the spiny fruit, which looks like a cocklebur, especially like the fruit of *Xanthium globosum*. In *Grossularia curvata* the 1-year-old twigs are dark reddish brown; the ovary bears sessile glands; the calyx tube is saucer-shaped, hardly more than a millimeter in length, much broader than long, and smooth or nearly so; the calyx lobes white; petals expanded and toothed; and fruit smooth. In the new species the filaments and style are only very sparingly hairy, in *curvata* densely and conspicuously hairy.

<sup>2</sup> Berries collected by Doctor Harper on May 10, though still hard, green, and unripe, had reached the astonishing diameter of 30 mm. (over 1½ inches), including the spines, and the body of the berry 22 mm. (over ¾ of an inch).

In the new species the sepals when reflexed reach to the summit or sometimes to the base of the ovary, in *curvata* far beyond its base.

On the pollination of *Grossularia echinella* only a few observations were made. Isolated plants did not set as much fruit as plants growing in groups and therefore better situated for cross-pollination. Only a single insect was seen pollinating the flowers, a carpenter bee, *Xylocopa virginica*. To suck the nectar it alighted in an inverted position on the pendent flower, grasping the stamens and style with all its legs in such a manner that the anthers and stigmas were brought into contact with the middle of the underside of its body, a very effective position for the transfer of pollen from one flower to another and from one plant to another. The bee assumed its inverted perch instantly, without hesitation and without slipping, drew the nectar rapidly, and proceeded promptly to another flower. In maintaining a firm grip on its perch it must have been greatly aided by the long hairs on the filaments of the flower, a characteristic very unusual in the genus *Grossularia*, and occurring in no other of the more than 40 American species except *curvata* and *nivea*. Both of these, like *echinella*, have long protruding stamens which, if devoid of hairs, would furnish only a slippery hold to a pollinating insect.

The cup-shaped glands at the tips of the coarse hairs that densely clothe the ovary were not yet yielding an exudate either in the newly opened flowers or in the older withered flowers in which the hairs had begun their elongation into prickles, a condition that suggested, at the time of the first observation of the inflorescence, that the function of the glands was related to the fruit, not to the flower. This suggestion was confirmed by the observations made on March 27 and by those made by Doctor Kurz on April 7 and later, when some of the berries were half mature. As the fruit enlarges, the glands at the ends of the prickles give off a sticky and unpalatable secretion. The species presents therefore the apparent anomaly of a sweet and succulent berry well adapted to the dispersal of its seeds by fruit-eating animals, yet barred from this means of dispersal by its spiny covering and offensive exudate. A little consideration, however, discloses the fact that these obstacles, though effective against such animals as insects, rabbits, and squirrels, would present only an interesting conundrum to such wide-awake and investigative fruit-eating animals as mocking birds, catbirds, and thrushes, whose long bills would enable them to open the berries with ease, and whose digestive limitations would insure a wide distribution of the living seeds. The structures that appear at first, therefore, as a bar against seed dispersal provide in all probability a very special and very effective means of advancing the distribution of the species.

At the present time the new species is known only from the type locality, on the north side of Lake Miccosukee, Fla. *Grossularia curvata* occurs in northern Georgia, northern Alabama, Louisiana, and eastern Texas. It may be questioned why a shrub so vigorous, so well protected against grazing animals, and so well adapted to dissemination by fruit-eating birds as *Grossularia echinella*, has such a limited geographical range, a strip of country about a mile in length and only a few rods in width. The manner of occurrence of the bushes in this area indicates that the establishment of the species here is of comparatively recent date. The plants occur in a definite center of abundance, with many individual bushes forming thickets, and farther away individual younger plants more widely separated from each other. The situation is exactly what would be expected if seeds of this species had been first introduced into this locality a few decades ago from some other and older center of distribution. The present known area of the species appears to be an advance colony, not a remnant, and it is to be expected that another and parent area will sometime be

discovered.<sup>3</sup> That the plants have not come from seeds scattered by birds from some foreign species cultivated in the neighborhood of Lake Miccosukee is evidenced by the fact that *Grossularia echinella* is very different from any of the species of Asia, Europe, or the mountains of northern Africa, the only parts of the world besides North America in which *Grossularias* are native.

The spininess of the fruit is the most remarkable characteristic of *Grossularia echinella*. Of the six other species of gooseberry native in eastern North America, *cynosbati*, *oxyacanthoides*, *hirtella*, *rotundifolia*, *missouriensis*, and *curvata*, only one, *cynosbati*, has spiny fruit, and that species is not closely related to the present species, as shown by their very different flower structure. Even in *cynosbati* the prickles of the fruit are comparatively few, in *echinella* they occur in hundreds. It is only in some of the Pacific coast species, such as *menziesii*, *hesperia*, and *hystrix*, that such dense spininess occurs, and the flower structure of all such spiny-fruited Pacific coast species shows that none of them is closely related to the new one.

*Grossularia echinella* is closely related to three other American gooseberries: *G. curvata*, of the southeastern United States; *missouriensis*, of the middle and upper Mississippi Valley region; and *nivea*, of the plains of eastern Washington and Oregon, western Idaho, and northern Nevada, a species which, though now stranded far from the others geographically, presents evidence of close genetic relationship with them, especially with *Grossularia curvata*. None of these species, however, has spiny fruit. But in *curvata* the ovaries are densely covered with sessile glands, and the elevation of these glands on stalks, a tendency which often appears in *Grossularia* and also in the related genus *Ribes*, provides a reasonable explanation of the evolution of the gland-tipped prickles of the new species.

In its geographic distribution *Grossularia echinella* is of special interest because it is the southernmost of our Atlantic seaboard species of this genus, growing in Florida, at an elevation of only about 200 feet above sea level, and in the principal region of production of the Satsuma orange. It may therefore be regarded as an almost subtropical representative of a north temperate genus. The cultivation of our present garden gooseberries in the latitude to which they are adapted, the Northern States, is now discouraged by forestry experts because the gooseberries, like the currants, are carriers of a blister rust that threatens the destruction of the white-pine forests. The danger is so seriously regarded that more than a million dollars has already been expended in the eradication of gooseberries and currants in the white-pine region. Should it be judged desirable that the agricultural range of the cultivated gooseberries be extended farther south than it is now possible to grow them, and that an attempt be made to establish gooseberry culture beyond the range of the white-pine forests, the new species offers a southern climatic adaptation which it may be possible to combine with the edible qualities of the garden gooseberries through hybridization. The culture of gooseberries in the southern coastal plain would carry no menace to the pine forests of that region because the hard pines are immune to the blister rust of the white pine, and, furthermore, the gooseberries in that region would not even have the disease because there are no white pines from which to contract it.

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<sup>3</sup> The prediction of a parent area has come true sooner than was expected. Doctor Kurz visiting Lake Miccosukee again on April 27 found that about a mile farther from Dogwood Landing the new species was running rampant as the dominant shrub of the forest belt and extending beyond it into the upland along the slopes of small streams emptying into the lake.

## PLATE 1

A.—Flowering branch. Natural size.

B.—Portion of a stem showing the cracked outer bark, and the stout nodal spines projecting backward. Natural size.

C.—Petal viewed from the inside of the pendent flower. Note the tubular form of the petal, brought about by the inrolling of the margins. A portion of a filament is shown beside the petal.  $\times 4$ .

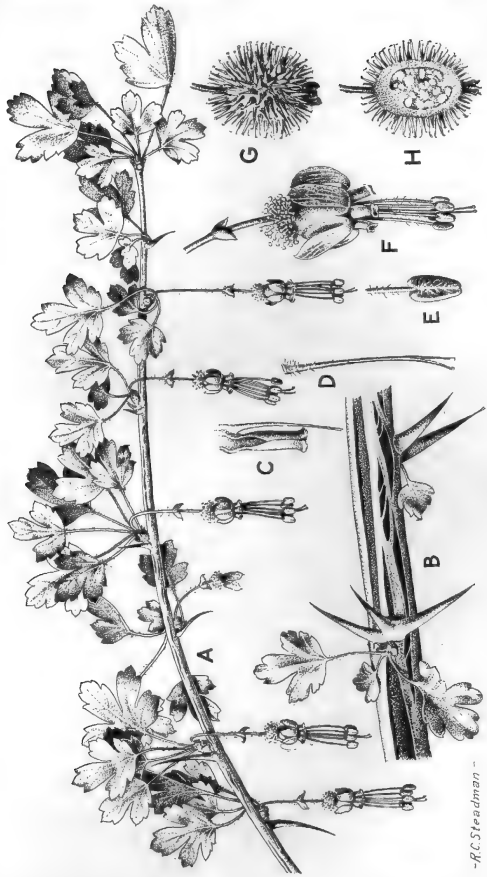
D.—Style.  $\times 2$ .

E.—Anther, from the back.  $\times 4$ .

F.—Flower, with pedicel, bracts, and portion of the peduncle. Note the hairy filaments, the tubular petals, the reflexed sepals, and the gland-tipped hairs of the ovary, which develop into the prickles of the fruit.  $\times 2$ .

G.—Immature berry, picked April 7. Natural size.

H.—Lengthwise section of an immature berry. Natural size.



-R.C. Steadman -



# FOWL TYPHOID, ITS DISSEMINATION AND CONTROL<sup>1</sup>

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## INTRODUCTION

The epornithological problems in the control of diseases of birds closely parallel the epidemiological problems in human beings. Intensive production of poultry in recent years has increased the problems of disease control, close study applied to this subject in the past few years bringing out the fact that even the closest system of quarantine leaves unexplainable loopholes for the spread of contagious poultry diseases.

Fowl typhoid undoubtedly stands out among the septicaemic diseases of the domestic fowl as a particularly virulent contagion. It is distributed throughout the various countries of the world, recognized as a particularly virulent disease caused by the *Bacterium sanguinarium* or *gallinarium* (*Eberthella sanguinaria* Moore), or, on the Continent, Klein's bacillus. As far back as 1888, Klein, in England, made an investigation of this disease, with such concise work as to have this specific bacterium known by his name in Europe. In 1895, Moore investigated the disease in the United States, calling it Infectious Leukemia of Fowls, and the causative organism the *Bacterium sanguinarium*. Since that time, the disease has been reported and investigated by various authorities.

## MORPHOLOGY

The fowl typhoid bacterium is a short, thick rod, occurring singly or in pairs, measuring 1 to 2 microns by 0.5 micron; stains peripherally; nonspore forming; Gram-negative, staining readily with most stains but best with fuchsin; non-motile.

## CULTURAL CHARACTERISTICS

Growth on agar slant beaded, abundant, raised, smooth, opaque in 24 hours; agar colonies raised, entire, circular, and finely granular; optimum temperature 37½° C.; optimum acidity of medium P<sub>H</sub> 6.4, although the organism has a high tolerance to organic acids, suitable growth being obtained on agar acidified with formic, malic, and oxalic acids between the ranges of P<sub>H</sub> 4.8 and P<sub>H</sub> 7.0; does not liquefy gelatin; nonchromogenic; heavy nitrate reduction in nitrate broth and on nitrate agar without gas; aerobic growth in glucose agar shake; slight production of hydrogen sulphid on surface of acetate agar; growth on potato fairly abundant and yellow-brown in color. There is a slight production in some instances of indol with the Salkowski test, but no reaction was obtained with the vanillin test, nor with Ehrlich's method; slight diastatic reaction takes place on starch agar; over 50 strains studied in 16 carbohydrates and higher alcohols showed no gas production at 37½° C. for 5 days. Acid is produced in

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<sup>1</sup> Accepted for publication Dec. 6, 1923.

all sugars with the exception of lactose, which remains neutral, with a slight tendency toward alkalinity; milk slightly acidified in 5 days. The thermal death point is  $62\frac{1}{2}^{\circ}$  for 10 minutes; the bacterium lives in both distilled and tap water in the dark for over 20 days, but is killed in the same medium in the sunlight in less than 24 hours; on glass rods the organism retains its vitality in the dark for up to 89 hours, but loses it in the sunlight in less than 30 hours; resists dry heat to the extent of giving good growth when subjected to  $75^{\circ}$  C. for 5 minutes, but fails to give growth when subjected to the same temperature for 10 minutes. Killed in dilution 1:1,000 phenol,  $\text{HgCl}_2$ , 1:20,000. Stock cultures show decided loss of virulence after being transferred several generations on artificial media.

### ARTIFICIAL INFECTION

Studies on 38 cases of typhoid, artificially infected per os, by subcutaneous inoculation, or through the drinking water, show the incubation period to be

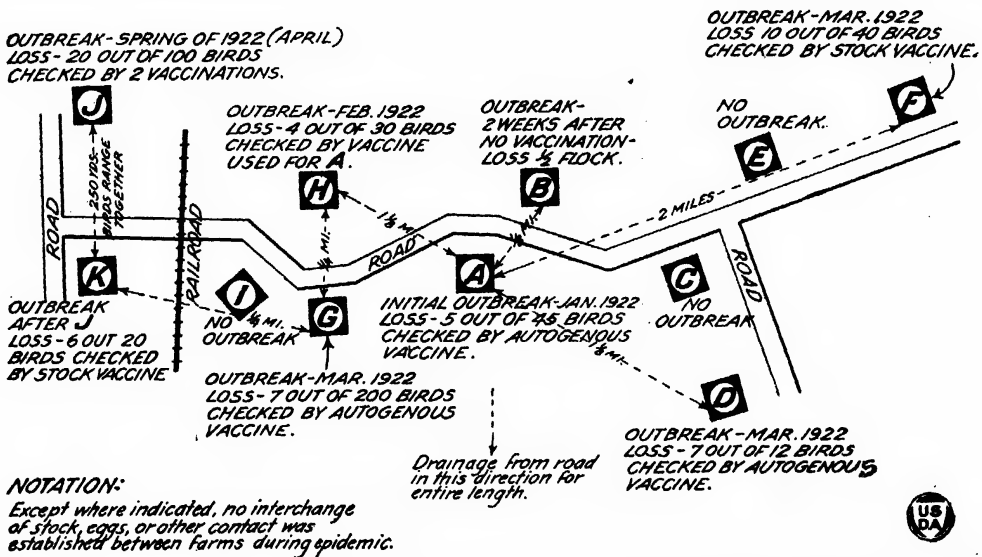


FIG. 1.—Map of region in which eight outbreaks of fowl typhoid occurred in the winter and spring of 1922.

from four to six days before definite clinical symptoms appear. Temperature in severe cases runs as high as  $111.5^{\circ}$ , respiration going as low as 23. The organism was recovered from the blood current of infected birds in two instances, four days after infection and six days after infection. In one instance the organism recovered was used as an infecting agent of another bird, this bird dying, while the original host recovered, having a normal laying rate until accidentally injured. There is a slight formation of agglutinins during the early part of the disease, demonstrated by Widal, and macroscopic agglutination tests in low dilutions. Hematological studies show a reduction of erythrocytes; leucocytosis, with a decided increase in the polymorphonuclear leucocytes. There is a decided lack of coagulability of the blood. The polymorphonuclear leucocytes may run as high as 95 per cent while the lymphocytes go down to 5 per cent; the erythrocytes fall as low as 1,160,000; the face, comb, and wattles become anemic; hemoglobin may fall to 75 per cent.

## CLINICAL STUDIES

In the course of investigation of this disease, the writers were consulted on numerous outbreaks in the territory adjacent to the institution. The outbreaks were marked by exceptional severity of infection, with an ensuing high death rate. In these outbreaks the disease usually affected the adults. Clinically the symptoms are not pronounced at first, the birds being dull, sleepy, showing a loss in appetite, with marked increase in thirst. There is a rise in temperature with a sulphurous discharge from the bowel which sometimes is whitish mucoid in character. Death is usually preceded by partial loss of use of limbs, dispnoea, subnormal temperature and profuse diarrhea. The duration of the disease depends on the severity of infection and the natural resistance of the bird. Some of the birds doubtless recover, to become carriers through virulent bacilli voided in the dejecta.

## GROSS AND MICROSCOPIC ANATOMY

Post mortem examinations show rigor mortis soon after death. The comb, face, and visible mucous membranes may be anemic. Serous effusion may be

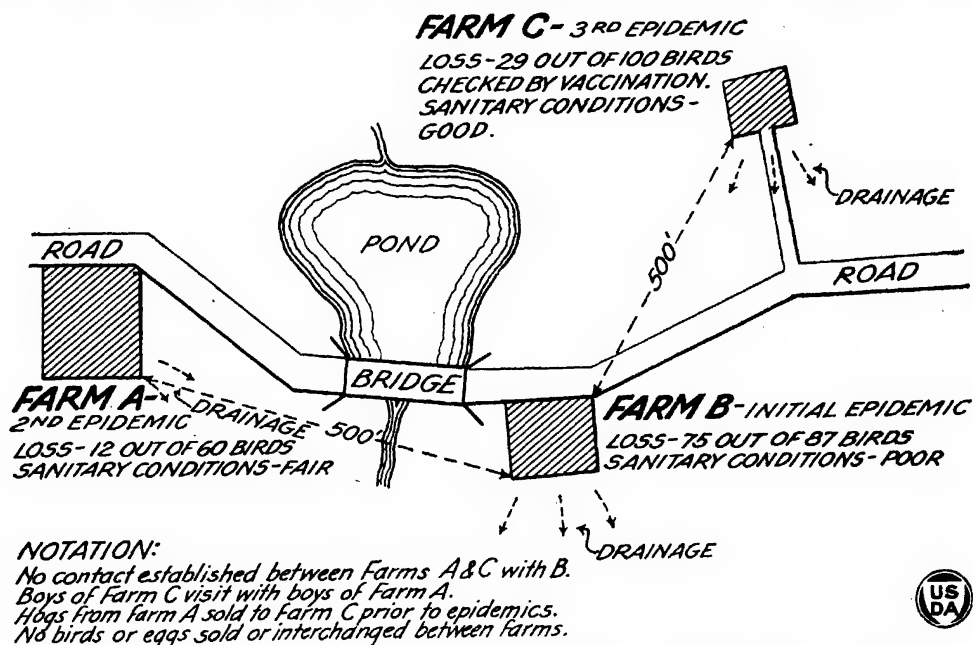


FIG. 2.—Map of region in which three outbreaks of fowl typhoid occurred.

observed around the heart and liver; hydropericardium may be present; the heart is congested and has a parboiled appearance; microscopically congestion is present, some of the muscle fibres have lost their cross striation, and cloudy swelling exists. The gross appearance of the liver shows enlargement, friability, dark-red in some cases, areas of focal necrosis exist, and blood drips from the sectioned surface; microscopically both active and passive congestion is present, areas invaded by cells of inflammation indicating hepatitis; cloudy swelling present; some areas show groups of cells losing their nuclei and nearing focal necrosis. The kidneys appear swollen, the sectioned surface is grayish in color, narrow red streaks are observed over the surface, indicating congestion; microscopically both active and passive congestion is present. Areas in which cells are losing their nuclei and nearing a state of focal necrosis are seen; glomerulitis may be present. The spleen is enlarged, dark, and sometimes mottled. The lungs are normal. *Eberthella sanguinaria* may be recovered from the heart, liver, spleen, kidneys, lungs, ovaries or testicles according to sex, bone marrow, and brain.

## EPIORNITHOLOGICAL STUDIES

As to the foci of infection, field studies in cases of well-defined epidemics have revealed in each case a starting point, with a definite advance of the disease. No conveying agent could be found, as only in rare instances could the investigators establish such contact between the locations of outbreaks as would be a factor in transmitting the disease. The possibility that English sparrows were the conveying agent is under investigation at the present time. Figures 1 and 2 show graphically the history of two typical epidemics of fowl typhoid. The progressive advance of the disease may be clearly traced.

## METHOD OF CONTROL

The most practical method of treating this disease is vaccination, using autogenous vaccines when possible, and stock vaccines when the former are not procurable. The same principle of desensitization as in making other vaccines is used in preparing fowl typhoid vaccine, 24-hour bacillary saline emulsion being heated one hour at 60° C., tested for efficiency of desensitization by pig inoculation and cultures, and preserved with one-half per cent phenol. The dosage used was 1 cc. for adults and one-half cc. for chicks, each weighing 1 to 2 pounds.

In 19 epidemics 2,140 birds were vaccinated. Prior to vaccination the loss had been 303 birds in these flocks; subsequent to vaccination the loss was 41, practically all of the birds lost having well-defined fowl typhoid when vaccinated. Of 974 birds prophylactically vaccinated this year on infected premises, no losses occurred from this disease.

# EFFECTS OF THE MODIFIED HOT-WATER TREATMENT ON GERMINATION, GROWTH, AND YIELD OF WHEAT<sup>1</sup>

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## INTRODUCTION

The loose smut of wheat (*Ustilago tritici* (Pers.) Jens.) (Pl. 1), according to estimates compiled in the Plant Disease Bulletin<sup>3</sup> for the years 1917 to 1921, inclusive, caused an average annual loss to farmers of the United States amounting to more than 11,000,000 bushels. Recommendations for control of this disease in this country have been confined to treatment of the seed by the modified hot-water method. In comparison with other standard cereal-seed treatments, however, this method is considerably more tedious to apply and also requires more labor and equipment, facts which doubtless help to explain why its adoption by the individual farmer has been limited. In 1918, R. G. East, county agricultural agent in Shelby County, Ind., in an attempt to make the modified hot water treatment more practicable, established a central or community seed-treatment plant at Shelbyville, Ind. The Division of Botany, Department of Agricultural Extension, of Purdue University, the Office of Cereal Investigations of the United States Department of Agriculture, and the Farmer's Association of Shelby County, Ind., cooperated with Mr. East in this endeavor. The community system became instantly popular and within two years, according to Pipal (10)<sup>4</sup>, 20 plants were operating in Indiana. A few plants also were in use in Virginia.

Pipal (10) has described the community system employed in Indiana, where two types of treating plant are used. One type operates in connection with a creamery, cannery, mill, or other establishment where a supply of live steam and suitable tanks are available. Here the farmers bring their grain and treat it in small quantities in sacks. The other type employs a special treating drum and tank for administering the 10-minute treatment, a device first used in 1918 by R. G. East. The drum is built of heavy, small-meshed wire or perforated sheet iron and is large enough to accommodate five bushels of soaked grain. It is equipped with a hoisting device operated by hand or motor. A crank also is provided to revolve the drum when it is lowered into the water. As it turns, the wheat tumbles over, thus insuring its uniform contact with the hot water. Following the treatment, the grain is spread out to cool; later it is resacked and returned to the farmer. In order to defray the expenses of the community plant

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<sup>2</sup> The writer makes grateful acknowledgment to Drs. W. H. Tisdale, H. B. Humphrey, and C. R. Ball for helpful criticisms of the manuscript, and to the agronomists in the wheat projects in the Office of Cereal Investigations for supplying many of the seed samples used in the experiments.

<sup>3</sup> U. S. Department of Agriculture, Bureau of Plant Industry, Plant Disease Survey. ESTIMATE OF CROP LOSSES DUE TO PLANT DISEASES, 1917. WHEAT. U. S. Dept. Agr. Bur. Plant. Indus. Plant. Dis. Bul. 2: 3-4. 1918. (Mimeographed.)

— CROP LOSSES FROM PLANT DISEASES IN THE UNITED STATES IN 1918-1921. WHEAT. U. S. Dept. Agr. Bur. Plant. Indus. Plant. Dis. Bul., Suppl. 6: 190-191; 12: 309-310; 18: 318-319; 24: 490-491. 1919-1922. (Mimeographed.)

<sup>4</sup> Reference is made by number (*italic*) to "Literature cited," p. 97.

a charge is made, based on actual cost of operation and equipment. The rate usually approximates 25 cents a bushel.

Table I, adapted from Pipal (10), shows the increase in number of community plants for applying the hot-water seed treatment in Indiana, and in the quantity of grain treated from 1918 to 1920.

TABLE I.—Increase in the number of community plants for applying the hot-water seed treatment in Indiana and in the quantity of grain treated from 1918 to 1920

Year	Type and number of treating plants			Bushels of wheat treated
	Using sacks in connection with a creamery, etc.	Using a treating drum	Total	
1918.....	0	1	1	616
1919.....	3	5	8	3,419
1920.....	11	9	20	9,687

This growth in the use of the modified hot-water treatment brought forth insistent inquiries concerning the effects of treatment on the germination, growth, and yield of wheat. Interviews with patrons of the community plants revealed that there was some apprehension regarding the amount of injury resulting from treatment. The available literature did not fully answer the questions and the investigations reported herein were undertaken to provide the desired information.

METHODS AND MATERIALS

In the following experiments the seed was treated in cheesecloth bags with ample room to allow for swelling of the grain. A 60-gallon tank, used for treatment, was equipped with two motor-driven propellers which kept the water thoroughly agitated and at a uniform temperature. Thermostatically controlled electric heaters were employed to maintain the temperature of the 10-minute bath, which was held at 54° C. in all cases with a plus or minus variation not in excess of 0.2°. All of the thermometers employed had been standardized by the United States Bureau of Standards. In applying the modified hot-water treatment the wheat was (1) presoaked for 4 to 5 hours in cold water, (2) dipped momentarily in water at about 49°, and (3) immersed in water at 54° for 10 minutes. Immediately after treatment the seed was spread in a thin layer to cool, and in order to reduce its moisture content to about that of the untreated seed, it was left to dry for 5 days or more at room temperature. Soil-germination tests in the greenhouse were made by sowing 100 seeds per flat 1 x 2 feet. Uniform spacing and depth of sowing were insured by pressing into the soil 100 one-inch pegs inserted in a board equidistantly. The kernels were dropped into the holes so made and then covered.

EFFECT OF THE MODIFIED HOT-WATER TREATMENT ON GERMINATION

Difficulties involved in making germination tests in the field throughout the year led to a study to determine whether or not tests in moist blotters or in soil in flats in the greenhouse could be substituted. Preliminary germination tests in moist blotters showed that many of the treated seeds germinated more or less abnormally. For instance, some of the primary rootlets would be stunted or absent, or only a plumule or a radical, or aborted forms of one or both would

appear. However, some of the seedlings which were abnormal at the end of the 5-day period usually allotted for the germination test assumed a normal appearance later on. In several instances 11 days were required for the germination of treated seeds to reach the stage commonly attained by untreated seeds on the fifth day. It was difficult or impossible to know whether or not seeds producing these types of seedlings in the blotters would produce plants if sown in the soil. An experiment was conducted, therefore, in order to study the effects of different periods of incubation on the percentage of germination in blotters and to compare the results from each period of incubation with those obtained from tests in soil in the field and in flats in the greenhouse. Five varieties were used, namely, Brown Winter Fife (C. I. 1933),<sup>5</sup> Currell (C. I. 3326), New Amber Longberry (C. I. 1973), Purplestraw (C. I. 1915) and Goens.<sup>6</sup> The Goens seed lot was grown in Indiana in 1921. The other varieties were grown on Arlington Experiment Farm, Rosslyn, Va., in 1921. A part of the seed of each of the varieties was treated on October 10, 1921, and dried 7 days at room temperature. Thereafter, 200 untreated and 200 treated seeds of each variety were taken for each of the soil tests and for the blotter test. All sowings were made on October 17, 1921. After 30 days germination counts were made in greenhouse and field and average percentages of germination for the 5 varieties were determined as follows:

Germination in soil in the greenhouse: Untreated seed 88.8 per cent; treated seed 66.9 per cent.

Germination in soil in the field: Untreated seed 89.2 per cent; treated seed 66.5 per cent.

A close agreement between field and greenhouse results is indicated. The results of the blotter test are presented in Table II. The numbers recorded at each germination period included only those seeds which appeared to germinate normally.

TABLE II.—Average percentages of germination of 200 untreated and 200 treated seeds of each of five wheat varieties sown in moist blotters

Treatment of seed	Varieties	Average percentage of germination after incubation for the following number of days:				
		3	5	7	9	11
Untreated.....	Brown Winter Fife.....	39.0	95.5	96.5	97.0	97.0
Do.....	Currell.....	53.0	96.5	98.0	98.0	98.0
Do.....	Goens.....	38.0	95.5	96.0	96.0	96.0
Do.....	New Amber Longberry.....	25.0	94.0	95.5	95.5	95.5
Do.....	Purplestraw.....	30.0	93.0	96.5	96.5	96.5
Average.....		37.0	94.9	96.5	96.6	96.6
Treated by the modified hot-water method.	Brown Winter Fife.....	0.5	63.0	89.0	90.5	91.5
Do.....	Currell.....	3.0	60.5	91.5	92.0	93.0
Do.....	Goens.....	2.5	64.5	85.5	86.0	86.0
Do.....	New Amber Longberry.....	0	49.5	82.5	84.5	84.5
Do.....	Purplestraw.....	1.5	61.0	87.5	88.0	88.5
Average.....		1.5	59.7	87.2	88.2	88.7

Table II shows that none of the periods of incubation yielded germination percentages which compared favorably with those obtained from seed sown in the soil. Treated seed, incubated seven days in the laboratory, germinated over 20 per

<sup>5</sup> Accession numbers of the Office of Cereal Investigations.  
<sup>6</sup> Goens wheat is extensively grown under its synonym, Red Chaff, in sections of Indiana where loose smut is very prevalent. It is unusually susceptible to loose smut, and perhaps more than any other variety is subjected to modified hot-water treatment. For these reasons it has been included in many of the experiments reported herein.

cent higher than treated seed sown in the soil. Evidently, many of the retarded or abnormal seedlings which survive on the blotter would not survive in the soil. The reliability of the test in greenhouse soil when compared with a field-soil test was confirmed in further trials and therefore was adopted in the germination studies which follow.

The effects of the modified hot-water treatment on germination were studied first on 33 varieties of wheat, 15 of which were grown on Arlington Experiment Farm, Va., and 18 at Chico, Calif., in 1921. A small portion of each of the varieties was treated on December 12, 1921, and dried six days at room temperature, after which 100 untreated and 100 treated kernels of each variety were sown in soil in greenhouse flats on December 18, 1921. Germination counts were made one month later and the results are presented in Table III. In this and other tables in which the wheat varieties are classified, the classification of Clark, Martin, and Ball (3) has been followed.

TABLE III.—Percentages of germination of machine-threshed seed of 33 wheat varieties, untreated or treated by the modified hot-water method

Variety	C. I. No.	Source	Percentage of germination	
			Un-treated	Treated
Hard red spring wheats:				
Haynes Bluestem.....	2874	Chico, Calif.....	92	44
Marquis.....	3641	do.....	86	60
Preston.....	3081	do.....	76	18
Durum wheats:				
Kubanka.....	1440	do.....	74	38
Monad.....	3320	do.....	77	46
Hard red winter wheats:				
Kanred.....	5146	do.....	93	40
Minturki.....	6155	do.....	77	12
Turkey.....	1558	do.....	92	47
Soft red winter wheats:				
Fulcaster.....	6162	Arlington Experiment Farm, Va..	90	53
Bearded Purplestraw.....	1911-1	do.....	94	57
Dietz.....	1981	do.....	97	70
Lancaster.....	1945	do.....	88	69
Stoner.....	2980	do.....	72	54
Fultz.....	3598	do.....	88	57
Harvest Queen.....	4882	do.....	92	27
Leap.....	4823	do.....	94	64
Mammoth Red.....	2008	do.....	87	57
Mediterranean.....	1930	do.....	88	60
Michigan Amber.....	4864	do.....	93	81
Minhardi.....	5149	Chico, Calif.....	90	40
Poole.....	3489	Arlington Experiment Farm, Va..	92	61
Red Rock.....	5976	do.....	73	40
Shepherd.....	6163	do.....	93	67
White wheats:				
Baart.....	1697	Chico, Calif.....	95	57
Dawson.....	6161	Arlington Experiment Farm, Va..	93	66
Dicklow.....	3663	Chico, Calif.....	92	51
Federation.....	4734	do.....	94	60
Genesee Giant.....	1744	do.....	77	62
Hard Federation.....	4733	do.....	92	76
Hybrid 128.....	4512	do.....	87	38
Little Club.....	4066	do.....	89	39
Pacific Bluestem.....	4067	do.....	93	69
Regenerated Defiance.....	3703	do.....	80	60
Average.....			87.6	52.7

Table III shows that the treated wheat in this experiment was injured severely, germinating 34.9 per cent less than the untreated, on an average.

During the experiment, it was observed that seed coats in many of the varieties were badly broken, and showed unmistakable signs of severe threshing injury. In this connection it is noteworthy that over 50 years ago Nobbe (?) pointed out that the unbroken seed coat is very effective in protecting the embryo



against injury from copper sulphate. He found that machine-threshed grain, on this account, was far more susceptible to treatment injury than grain threshed by hand. Kühn (6), Von Tubeuf (13), Volkart (14), Burmester (2), Hurd (5), and others have confirmed Nobbe's work. A test was made, therefore, to determine whether the physical condition of the seed coat might be an important factor also in connection with seed injury by the modified hot-water treatment. Three hand-threshed wheat varieties, grown on Arlington Experiment Farm in 1921, were used. Four lots, each containing 100 kernels, were taken from each variety and prepared as follows: Lot 1, seed coats left unbroken, seed untreated; lot 2, seed coats left unbroken, seed treated; lot 3, seed coats slit over the embryo, seed untreated; lot 4, seed coats slit over the embryo, seed treated. (The seed coat may be slit easily with a sharply pointed needle after first immersing the kernels in water for several minutes.) The modified hot-water treatment was applied simultaneously to lots 2 and 4 on January 25, 1922, after which they were dried six days at room temperature. The untreated and treated seed was sown then in soil in the greenhouse on January 31, 1922. One month later the seedlings were counted. The results are presented in Table IV.

TABLE IV.—*Percentages of germination of hand-threshed seed of three wheat varieties with seed coats unbroken or broken over the embryo, untreated or treated by the modified hot-water method*

Variety	C. I. No.	Percentage of germination			
		Seed coat unbroken		Seed coat broken over embryo	
		Untreated	Treated	Untreated	Treated
Fulcaster.....	3115-2	100	100	100	6 <sup>a</sup>
Dawson.....	1733	98	100	98	4 <sup>a</sup>
Diehl-Mediterranean.....	1395	96	94	97	3 <sup>a</sup>
Average.....		98.0	98.0	98.3	4.3

<sup>a</sup> Seedlings very small and spindling.

As indicated in Table IV, the hot-water treatment reduced germination only when the seed coats were broken. The physical condition of the seed coat over the embryo, therefore, bears a definite and important relation to the amount of injury caused by the modified hot-water treatment (Pl. 2).

In many samples of machine-threshed wheat, breaks in the seed coats frequently were found over the endosperm only. In order to determine the effects of hot-water treatment on grain with seed coats in this condition, four lots of 200 kernels each from a hand-threshed sample of the variety China (C. I. 180) were used. The seed coats were left unbroken in the first lot. In the second lot, a small slit was made in the coat about one-eighth of an inch above the top of the embryo. In the third lot, a small part of the seed coat was scraped away at the brush end of the seed. In the fourth lot, a slit was made in the seed coat over each cheek in a location behind the embryo. One hundred kernels of each of the four lots were treated simultaneously on September 4, 1922, and dried 5 days at room temperature. The untreated and treated seed was then sown in soil in greenhouse flats on September 9, 1922, and the seedlings were counted after periods of 10, 20, and 30 days. Many which developed from the treated seed with the coats broken over the endosperm were very small and spindling. For this reason, the seedlings were recorded as either normal or abnormal at each of the three periods when the counts were taken. The results are presented in Table V.

TABLE V.—Percentages of normal, abnormal, and total germination of untreated and treated seeds of China (C. I. 180) wheat with seed coats unbroken or broken at definite locations over the endosperm

Number of days after sowing	Percentage and character of germination															
	Seed coats unbroken				Seed coats broken over endosperm											
					$\frac{1}{2}$ inch above embryo				On brush end				On each cheek behind embryo			
	Un-treated		Treated		Un-treated		Treated		Un-treated		Treated		Un-treated		Treated	
	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
10.....	97	0	93	0	99	0	15	40	95	0	9	15	98	0	2	3
20.....	97	0	96	0	99	0	15	49	95	0	9	26	98	0	4	7
30.....	97	0	96	0	99	0	15	51	95	0	9	27	98	0	4	8
Total germination.....	97		96		99		66		95		36		98		12	

Table V shows that when the seed coats are broken over the endosperm there is a severe reduction in normal and total germination (Pl. 3). In the light of these data, the original supply of the 33 varieties listed in Table III was examined carefully for broken seed coats. It was found that the lots most severely injured by treatment invariably showed a high percentage of seed-coat breakage.

During the course of some of the preceding experiments it was observed that hand-threshed kernels with unbroken seed coats germinated more slowly when the seed was treated. A typical example is shown in Table VI for Leap (C. I. 4823) wheat. Five hundred hand-threshed kernels were treated on September 1, 1922, and dried seven days at room temperature, following which, the treated kernels and a like number of untreated from the same lot were sown on September 8, 1922, in soil in greenhouse flats. All of the seed was sown and germinated under like conditions. From the date of emergence of the first seedling daily records of the number of seedlings which had emerged were taken for one month. The results are presented in Table VI.

TABLE VI.—Rate of emergence of seedlings from 500 untreated and 500 treated kernels of hand-threshed Leap (C. I. 4823) wheat

Treatment of seed	Percentage of emergence after the following number of days						
	5	6	7	8	9	10	30
Untreated.....	85	98	98	98	98	98	98
Treated by the modified hot-water method.....	0	22	64	79	96	98	98

Table VI shows that the modified hot-water treatment materially affects the germination of seeds with unbroken coats, although it may produce no actual mortality (Pl. 4, A, B, E, and F). Atwood (1) has noted the possibility that concentrations of formaldehyde which do not materially injure germination percentages may materially disturb the physiologic processes related to germination and subsequent growth.

It is noted thus far that the action of the modified hot-water treatment on germination may range from retardation without reduction to outright killing of practically 100 per cent of the seed, depending entirely upon the physical condition of the seed coat. In actual practice seed-coat damage results mainly from threshing. An indication of the amount of possible seed injury traceable to the modified hot-water treatment following threshing injury to seed coats has been obtained from 25 samples of the crop of 1922. Twenty of them came from Hancock, Rush, and Shelby Counties, Ind., where loose smut usually occurs abundantly, and where the hot-water treatment is used extensively. Each of the 25 seed lots consisted of both machine-threshed grain and grain in the head taken from the field on the day of threshing. The grain in the head was carefully threshed by hand. One hundred kernels of each of the 25 different lots of hand-threshed and of machine-threshed seed were treated simultaneously, on October 14, 1922. The grain then was dried six days at room temperature and sown on October 20 in soil in greenhouse flats at Arlington Experiment Farm. One hundred untreated hand-threshed kernels and 100 untreated machine-threshed kernels of each seed lot were sown in similar flats as controls. The seedlings were counted on November 14, one month after sowing. The results are presented in Table VII and in Plate 4.

TABLE VII.—*Percentages of germination of wheat showing the effects of the modified hot-water treatment on machine-threshed and hand-threshed seed of 25 different lots of seed from nine varieties as compared with untreated controls*

Class and variety	Source	Seed lot	Percentage of germination			
			Machine-threshed		Hand-threshed	
			Un-treated	Treated	Un-treated	Treated
Soft red winter wheats:						
Fultz.....	Indiana.....	A	85	61	99	95
Do.....	do.....	B	96	68	96	95
Goens (Red chaff).....	do.....	C	90	40	98	95
Do.....	do.....	D	99	71	98	98
Do.....	do.....	E	89	58	100	100
Do.....	do.....	F	94	62	98	99
Do.....	do.....	G	90	52	99	97
Do.....	do.....	H	81	49	99	99
Do.....	do.....	I	88	64	99	99
Do.....	do.....	J	89	52	96	96
Do.....	do.....	K	71	45	99	97
Do.....	do.....	L	85	55	100	98
Do.....	do.....	M	90	51	99	98
Leap.....	Virginia.....	N	78	39	97	97
Poole.....	Indiana.....	O	87	54	97	96
Do.....	do.....	P	93	64	98	96
Do.....	do.....	Q	87	45	100	99
Red May (Michigan amber).....	do.....	R	93	77	99	100
Do.....	do.....	S	90	65	100	96
Do.....	do.....	T	99	77	99	100
Do.....	do.....	U	89	61	100	93
White wheats:						
Defiance.....	Idaho.....	V	91	53	100	99
Dicklow.....	do.....	W	84	34	100	97
Hard Federation.....	do.....	X	84	61	82	83
Little Club.....	California.....	Y	86	57	96	99
Average.....	.....	.....	88.3	56.6	97.9	96.8

Table VII shows a reduction of 31.7 per cent in the germination of the treated machine-threshed seed, while the treated hand-threshed seed was reduced only 1.1 per cent. This difference of 30.6 per cent between the germination of machine-threshed and hand-threshed seed shows clearly the relation of mechanical injury of the seed coat and possible subsequent injury from application of hot water (Pl. 4).

Having shown before that unbroken seed coats protect the grain from treatment injury, it may be asked why treated hand-threshed wheat (Table VII) did not germinate fully as well as untreated hand-threshed seed. It is possible that some injury to seed coats may result even from hand threshing. Also, localized imperfections too slight even to be seen microscopically, may occur in seed coats. Shull (12) found such a condition to be frequent in the seed coats of *Xanthium*. Hurd (5) observed that wheat kernels with apparently unbroken seed coats occasionally were injured when treated with copper sulphate.

In an experiment to determine if a break in the seed coat invariably could be detected microscopically and thus enable the observer to predict the amount of injury likely to result from treatment, a machine-threshed sample of Haynes Bluestem (C. I. 2874) was employed. The untreated seed germinated 95 per cent. One hundred kernels selected at random and treated, germinated 12 per cent, while a like number of kernels microscopically examined, selected for unbroken seed coats, and treated simultaneously with the above, germinated 74 per cent. This indicates that it is hardly possible to detect all of the breaks in a seed coat even under the microscope, and that the small fractures which escape detection may result fatally to the embryo through injury from treatment. Wallden (15) was able to detect broken seed coats by the use of eosin, which does not penetrate the unbroken seed coat, but enters every small fissure, staining the seed tissues at the point of entrance.

#### THE NATURE OF SEED-COAT PROTECTION FROM HOT-WATER INJURY

The important rôle of the seed coat in protecting the embryo from injury during the hot-water treatment led to an investigation of the manner in which it functions. Obviously, this protective action must be exerted either in the 4 to 6-hour presoak in cold water, in the 10-minute bath in water at 54° C., or in both. In an experiment to determine the point, three lots of 50 hand-threshed kernels of each of the three varieties, Leap (C. I. 4823), Poole (C. I. 3489), and Power Fife (C. I. 3697) were used. In the first lot the seed coats were left unbroken through both the presoak and 10-minute baths. In the second lot the seed coats were broken over the embryo before the 10-minute bath. In the third lot the seed coats were broken over the embryo before both baths. The presoak bath for 5 hours at 20° C. was applied to all lots simultaneously on February 10, 1923, and each lot was weighed carefully before and after presoaking to determine the possible influence of broken seed coats on the amount of water absorbed by the seed. All lots were treated then simultaneously for 10 minutes at 54° C., dried 6 days at room temperature, and then sown on February 16, 1923, in soil in greenhouse flats. One month later, on March 16, the seedlings were counted. The results are presented in Table VIII.

TABLE VIII.—Effect of the presoak bath or 10-minute bath, or both, on wheat with seed coats unbroken or broken during the 10-minute bath or throughout treatment

Variety	C.I.No.	Actual and average percentage of germination when seed coats were:		
		Unbroken throughout treatment	Unbroken during presoak bath; broken during 10-minute bath	Broken throughout treatment
Leap.....	4823	81.0	14.0	0
Poole.....	3489	98.0	34.0	0
Power Fife.....	3697	100.0	24.0	0
Average.....		93.0	24.0	0
Average percentage of increase in weight at end of presoak bath.....		23.09	23.04	27.65

Table VIII shows that germination was increased from zero to 24 per cent when seed coats were unbroken during the presoak and from 24 to 93 per cent when seed coats were unbroken throughout treatment. Unbroken seed coats, therefore, effect protection from injury during both baths of the modified hot-water method and particularly during the 10-minute treatment. Table VIII also shows that the amount of water absorbed by the seed was greater in the presence of seed coats broken during the presoak bath.

According to Osborne (8) leucosin is the only one of three proteins in the wheat embryo affected by a temperature of 54° C. A solution of leucosin (after a 4 to 6-hour presoak, the leucosin of the embryo undoubtedly is in solution) becomes turbid at 48°, and forms a flocculent coagulum at 55°. The hot water at 54°, therefore, through direct contact with the embryo, probably coagulates some of the leucosin when the surrounding seed coat is broken, and thus may bring about the lethal action previously noted.

An examination of its physical structure shows how the seed coat may function to insulate the embryo. According to Percival (9) and others, the outermost membrane of the seed coat over the embryo is the pericarp, and the inner membranes successively are the testa and nucellar layer. It was found that the presence or absence of the pericarp did not influence the germination of hand-threshed treated seeds. The pericarp was removed <sup>7</sup> from 100 hand-threshed kernels of Fulcaster (C. I. 6162). A like number of kernels from the same lot with unbroken pericarps were employed. The two lots of seed were subjected to the hot-water treatment simultaneously and each was sown in soil in greenhouse flats. The resulting germination was 100 per cent for each lot. Hurd (5) found that the presence or absence of the pericarp also had no influence on the germination of hand-threshed seeds treated with copper sulphate. The burden of protection, therefore, falls upon the testa or the nucellar layer, or both. According to Percival (9), the crushed cells of these membranes in the ripe seed together form a layer six cell-walls thick over the embryo, and it seems not unreasonable that a structure of this nature might afford protection to the extent noted during the 10-minute treatment period.

Table VIII also shows that some protection was afforded by unbroken seed coats during the presoak, and that the rate of water absorption by the seed during the presoak was greater when the seed coats were broken. In order to determine

<sup>7</sup> Soaking the seed in water for several minutes causes the pericarp to pucker and loosen, when it may be peeled off readily with the aid of a needle or scalpel.

the relationship between water content of the seed and injury by treatment in the presence of broken and unbroken seed coats, several experiments were conducted. In the first, 8 lots each containing 100 hand-threshed kernels of the variety Haynes Bluestem (C. I. 2874) were used. In 5 of the lots the seed coats were broken over the embryo with a needle. Some of the lots were presoaked for varying periods, as shown in Table IX, and a record was made of the resultant increases in weight. The seed coats were undisturbed in three of the lots. All of the seed was subjected simultaneously to the 10-minute treatment at 54° C., on February 15, 1923, then dried five days at room temperature, and sown in flats in the greenhouse on February 20. The seedlings were counted on March 20 and the results are presented in Table IX and in Plate 5.

TABLE IX.—Percentages of germination and increase in weight of hand-threshed seed of Haynes Bluestem wheat (C. I. 2874) with broken or unbroken seed coats, some presoaked during different periods prior to treatment with hot water

Lot No.	Condition of seed coat	Length of presoak bath	Weight of seed before presoak bath	Weight of seed after presoak bath	Increase in weight	10-minute treatment at 54° C	Germination
		Hours	Gm.	Gm.	Per cent		Per cent
1.....	Broken over the embryo	0	.....	.....	.....	Untreated.....	96.0
2.....	do.....	0	.....	.....	.....	Treated.....	38.0
3.....	do.....	1	3.260	3.745	14.9	do.....	19.0
4.....	do.....	2	3.180	3.860	21.4	do.....	15.0
5.....	do.....	4	3.250	4.130	27.1	do.....	4.0
6.....	Unbroken.....	0	.....	.....	.....	Untreated.....	95.0
7.....	do.....	8.5	3.370	4.510	33.8	Treated.....	96.0
8.....	do.....	10.5	3.380	4.660	37.9	do.....	93.0

Table IX shows (1) that in the absence of the presoak bath severe reduction in germination resulted from the 10-minute treatment when the seed coats were broken over the embryo (lot 2); (2) that injury increased with increase in duration of presoak and moisture content when the seed coats were broken over the embryo (lots 3, 4, and 5); and (3) that treatment did not impair germination appreciably when the seed coats were unbroken despite a high increase in water content (lots 7 and 8), (Pl. 5).

The relationship between increase in the duration of the presoak period and injury to seed with unbroken coats was studied further in the experiment which follows. Three lots each containing 50 hand-threshed seeds were prepared from each of four varieties of wheat. The three lots from each variety were presoaked 5, 10, and 15 hours, respectively, on February 18, 1923, then all were treated simultaneously in the 10-minute bath at 54° C., dried at room temperature for one week, and sown on February 25 in soil in greenhouse flats. The germination counts were made after 10, 15, and 30 days. The results are presented in Table X.

TABLE X.—Percentages of germination of three lots of 50 hand-threshed seeds of each of four varieties of wheat presoaked for 5, 10, and 15 hours, respectively, prior to treatment for 10 minutes in water at 54° C.

Variety	C. I. No.	Percentage of germination after:								
		10 days			15 days			30 days		
		Hours presoaked			Hours presoaked			Hours presoaked		
		5	10	15	5	10	15	5	10	15
Forward.....	6691	94	80	30	96	92	74	96	96	78
Goens.....		80	28	2	84	58	26	84	72	32
Illini Chief.....	5406	100	82	38	100	96	64	100	98	80
Stoner.....	2980	88	38	26	92	64	68	92	70	78
Average.....		90.5	57.0	24.0	93.0	77.5	58.0	93.0	84.0	67.0

Table X shows that even in the presence of unbroken seed coats increases in the duration of presoaking slowly result in increased retardation and a further reduction in germination.

#### EFFECTS OF THE MODIFIED HOT-WATER TREATMENT ON GROWTH

The retardation in germination and emergence of seedlings from kernels which survive treatment has been noted. The effects of treatment on seedling growth also were studied. Five lots of 100 seeds each were prepared, three lots being Goens (Red Chaff) and one lot each being Fulcaster and Purplestraw. Fifty seeds from each lot were subjected to modified hot-water treatment on October 14, 1921, and then dried for one week. On October 21 all of the seeds were sown in soil in greenhouse flats. Eleven days later, on November 1, the height of each seedling was recorded. The results are presented in Table XI.

TABLE XI.—Average height, 11 days after sowing, of seedlings from five lots of wheat grown from untreated seed and seed treated by the modified hot-water method

Variety	Average height of seedlings in inches 11 days after sowing	
	Seed untreated	Seed treated
Goens.....	4.50	2.25
Do.....	4.75	2.25
Do.....	5.50	3.25
Fulcaster.....	5.00	4.00
Purplestraw.....	4.75	3.25
Average.....	4.90	3.00

Table XI shows that the seedlings from the treated half of the five lots averaged 1.9 inches shorter than the seedlings from untreated seed.

To facilitate a critical determination of the effects of the modified hot-water treatment on the growth of wheat from treated seed, studies were made of the effects of treatment on individual plants on (1) field germination, (2) overwintering, and (3) number of culms produced. The determination of the plant



yields included in the plan of experiment had to be abandoned. One hundred untreated and 100 treated machine-threshed kernels of each of the varieties, Fulcaster (C. I. 6162) and Purplestraw (C. I. 1915), grown on Arlington Experiment Farm, in 1921, were used. The treatment was applied on October 14, 1921, and the seed allowed to dry five days at room temperature. On October 19 the untreated and treated kernels of each variety were sown in the field in adjacent sets of 100 seeds, and each set consisted of three successive rows containing 33, 33, and 34 kernels, respectively. The kernels were spaced 7 inches apart in rows 1 foot apart.<sup>8</sup> One month after sowing, the seedlings were counted. In July, 1922, the number of plants which had matured and the average number of culms per plant were determined. The results are presented in Table XII.

TABLE XII.—*Effects of the modified hot-water treatment on the development of wheat plants from machine-threshed seed*

Variety	Seed untreated			Seed treated		
	Field germination	Plants overwintered and matured	Average number of culms per plant	Field germination	Plants overwintered and matured	Average number of culms per plant
Fulcaster.....	<i>Per cent</i> 90.0	<i>Per cent</i> 90.0	12.0	<i>Per cent</i> 71.0	<i>Per cent</i> 71.0	11.6
Purplestraw.....	79.0	79.0	19.3	76.0	76.0	19.1
Average.....	84.5	84.5	15.7	73.5	73.5	15.4

Table XII shows (1) that the field germination was somewhat less and that the number of culms per plant was slightly less from treated than from untreated wheat and (2) that plants from untreated and treated seed overwintered without reduction in numbers. The fact that the number of culms produced per plant was less from treated seed than from untreated seed suggests the possibility that the injurious effects of treatment on spaced plants may extend throughout their life, if we assume that the culms from treated seed did not produce heads of greater weight. Repetition of the experiment on a larger scale would be desirable, however, before drawing definite conclusions.

#### EFFECTS OF THE MODIFIED HOT-WATER TREATMENT ON YIELD

Experiments to determine the effects of the modified hot-water treatment on yield have been conducted for three years. On October 19, 1920, definite quantities of each of two lots of Goens (Red Chaff) wheat from different sources in Indiana were weighed and measured, treated, spread out in thin layers and left to dry for one week. At the end of this period the treated seed had regained its original weight and volume. On October 26, the untreated and treated seed of both lots was sown in fortieth-acre plats on Arlington Experiment Farm, Va., at the rate of 6 pecks per acre. The results are presented in Table XIII.

<sup>8</sup> Unpublished data by Dr. C. E. Leighty seem to indicate that at this distance the effect of competition by wheat plants is nil.



TABLE XIII.—Loose smut infection and yield in 1921 of two lots of Goens wheat grown from untreated seed and from seed treated by the modified hot-water method

Lot number	Acre yield in bushels		Percentage of loose smut	
	Untreated	Treated	Untreated	Treated
7.....	25.2	24.1	11	0
13.....	25.0	24.2	7	0
Average.....	25.10	24.15	9	0

Table XIII shows that plants from treated seed yielded 0.95 of a bushel per acre less than those from untreated, although the latter contained an average of 9 per cent loose smut.

In a yield test in 1922, there were used three different lots of Goens wheat grown in Indiana in 1921, and one lot each of Fulcaster (C. I. 6162) and Purplestraw (C. I. 1915) wheats grown on Arlington Experiment Farm in 1921. A definite quantity of each lot was weighed and measured, treated on October 13, 1921, spread out in thin layers, and dried for one week. At the end of this period the treated seed had regained its original weight and volume. On October 20 the untreated and treated seed was sown in rod rows on Arlington Experiment Farm, Va., at the rate of 6 pecks per acre on the same land used for the yield experiments of the preceding year. Five plats, each divided into 2 sections, were used. Each of the 10 sections contained 55 rows, or 11 successive rows of each of the five seed lots. The first 9 rows of each seed lot comprised a study of other hot-water treatments not discussed in this paper, while the tenth and eleventh were sown to seed treated by the modified method and to the untreated seed, respectively. The results are presented in Table XIV.

TABLE XIV.—Loose smut infection and yields in 1922 of five lots of wheat each grown from untreated seed and from seed treated by the modified hot-water method

Section of plat	Lot No.	Variety	Acre yield in bushels of each of 5 plats										Average percentage of loose smut for all 5 plats	
			Plat 1		Plat 2		Plat 3		Plat 4		Plat 5			
			Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
East.....	16	Goens.....	35.1	29.0	35.0	27.7	40.7	30.0	31.3	25.3	35.4	24.0	P. c.	P. c.
Do.....	17	do.....	34.5	20.8	35.1	19.5	37.3	24.5	33.5	20.8	32.1	20.0	7.2	0
Do.....	19	do.....	33.3	23.3	32.4	26.6	31.0	21.4	29.7	24.8	32.0	22.7	6.8	0
Do.....	26	Fulcaster.....	22.7	24.6	23.8	21.9	25.1	22.3	27.7	16.8	26.8	22.0	10.9	0
Do.....	27	Purplestraw.....	28.6	17.3	36.3	26.5	44.1	30.5	39.3	26.1	38.1	28.5	0	0
Average.....			30.8	23.0	33.5	24.4	35.6	25.7	32.3	22.8	32.9	23.4	.....	.....
West.....	16	Goens.....	32.1	28.7	36.3	26.8	40.5	29.3	38.1	33.6	35.3	34.3	7.1	0
Do.....	17	do.....	36.0	23.8	40.1	31.6	39.5	25.6	39.7	25.7	34.5	24.3	11.1	0
Do.....	19	do.....	31.1	28.6	29.7	28.3	32.8	33.9	32.1	27.3	28.0	21.1	15.0	0
Do.....	26	Fulcaster.....	32.0	32.0	28.0	29.4	31.8	33.3	34.8	32.7	31.4	24.3	0	0
Do.....	27	Purplestraw.....	30.6	27.4	34.6	31.0	34.8	35.7	36.6	29.7	37.0	32.2	0	0
Average.....			32.4	28.1	33.7	29.4	35.9	31.6	36.3	29.8	33.2	27.2	.....	.....

Average yield for all plats, east section: Untreated 33.0 bushels per acre; treated 23.9 bushels per acre.

Average yield for all plats, west section: Untreated 34.3 bushels per acre; treated 29.2 bushels per acre.

Average yield for all plats, both sections: Untreated 33.7 bushels per acre; treated 26.6 bushels per acre.

Table XIV shows that although the plants from the untreated seed contained an average of 5.8 per cent of loose smut, they outyielded those from the treated seed by 7.1 bushels per acre. The average from 7 seed lots in the two years shows that treated seed yielded 4 bushels per acre less than untreated, although plants from the latter contained 7.4 per cent of smutted heads.

A comparison of the yields for the two years will show that reduction in yield from treated seed in 1922, amounting to 7.1 bushels per acre, is approximately 7.5 times the reduction of 0.95 bushels per acre in the yield from treated seed in 1921. It has been shown before (Tables IV and V) that the amount of seed injury resulting from the modified hot-water treatment is intimately related to the percentage of seed coats broken in threshing. Investigators have shown, moreover, that the amount of damage suffered by seed coats in the process of threshing is markedly greater in dry years than in wet years. Nobbe (?) first pointed out that the drier and more brittle the crop, the greater was the injury from threshing. Volkart (14) made similar observations and Wallden (15) also noted that in dry years the cracking of seed coats by threshing machines was more prevalent. In this connection, therefore, a study was made of the climatologic data for June and July, in 1920 and 1921, for the localities which supplied the seed used in the yield experiments in 1921 and 1922, respectively. The seed used was ripened, harvested, and threshed during June and July and the effects of weather conditions on the seed coats occurred in those months. The climatologic data are presented in Table XV.

TABLE XV.—*Temperature and rainfall during June and July, 1920 and 1921, in the localities in which was grown the seed wheat used in the yield experiments of 1921 and 1922, respectively*

County	State	Lot number	Departures from normal				
			Year	Temperature		Rainfall	
				June	July	June	July
				° F.	° F.	Inches	Inches
Hancock.....	Indiana.....	7	1920	-1.0	-1.7	+1.52	+1.29
Shelby.....	do.....	13	do...	-1.0	-2.1	-0.61	0
Rush.....	do.....	16, 17	1921	+2.4	+4.9	-0.66	-0.10
Hancock.....	do.....	19	do...	+5.9	+5.4	-1.12	+0.30
Arlington.....	Virginia.....	26, 27	do...	+1.7	+2.4	-0.73	+0.14

Reference to Table XV shows abnormally low temperatures and a rainfall slightly above normal in June and July, 1920, in the localities which supplied the seed used in 1920-21. For the State of Indiana, as a whole, July, 1920, was one of the coolest on official record. In striking contrast, the temperatures were abnormally high and the rainfall below normal in June and July, 1921, in the counties supplying the seed used in 1921-1922. June, 1921, was the warmest on official record in Indiana and the average temperature of July, 1921, has been exceeded only twice in 34 years. It was the hottest July in Virginia since 1901. In the light of these data, the condition of the seed coats of wheat grown in 1920 and 1921 was examined carefully. It was very evident that the latter had sustained considerably more injury in threshing. It is interesting and noteworthy that the weather conditions, through their influence on the seed coats in withstanding threshing injury, may be potent factors in determining the amount of seed injury and subsequent yield which result from treatment of the seed by the modified hot-water method.

Attention is directed to the parts of Table XIV dealing with the average yields on the east and west sections of the plats. It will be noted that the

average yields per plat from both the untreated and treated seed were higher on the west than on the east sections, without exception. It is noteworthy, however, that the average yield of all the plats of the west section exceeded that of the east section by only 1.3 bushels per acre when the seed was untreated and by over four times this amount (5.3 bushels per acre) when the seed was treated. This shows a pronouncedly stronger reaction to less favorable soil conditions in plants from treated seed.

In the yield tests of 1923, part of the treated seed was sown at an increased rate to offset the loss in stand brought about by the treatment. About a month before the usual time of sowing (September 15, 1922) small quantities of each of two seed lots of Goens wheat from different sources in Indiana were treated and dried one week at room temperature. Subsequently, 500 untreated and 500 treated kernels of each seed lot were sown (September 22, 1922) in the field which was used for the later sowings. Three weeks later (October 13, 1922), germination counts were made with the following results:

Seed lot 31 from untreated seed, 87.0 per cent; from treated seed, 48.0 per cent.

Seed lot 35 from untreated seed, 84.5 per cent; from treated seed, 54.5 per cent.

In view of the fact that germination of both lots of treated seed was approximately 50 per cent, the rate of seeding was doubled in part of the sowings. Definite quantities of each of the two seed lots were weighed and measured, treated on October 18, 1922, spread out in thin layers and dried one week. At the end of this period, the treated seed had regained its original weight and volume. All of the untreated and part of the treated seed was sown then on October 25, in rod rows at the rate of 6 pecks per acre. Part of the treated seed was sown at the rate of 12 pecks per acre. In order to determine the effects of sowing freshly treated seed, a practice carried on by some of the patrons of the community seed-treating plants for the purpose of saving the labor and time involved in drying grain, seed from both lots was treated and sown wet at the rates of 6 and 12 pecks per acre, respectively, also on October 25. The necessary amount for each row was weighed and treated in a small gauze sack. The order of sowings in the plat was as follows in 10 successive replications.

*Seed lot No. 31.*—Seed untreated; sown dry; 6 pecks per acre. Seed treated; sown dry; 6 pecks per acre. Seed treated; sown dry; 12 pecks per acre. Seed treated; sown wet; 6 pecks per acre. Seed treated; sown wet; 12 pecks per acre.

*Seed lot No. 35.*—Seed untreated; sown dry; 6 pecks per acre. Seed treated; sown dry; 6 pecks per acre. Seed treated; sown dry; 12 pecks per acre. Seed treated; sown wet; 6 pecks per acre. Seed treated; sown wet; 12 pecks per acre.

The results are presented in Table XVI.

TABLE XVI.—Loose smut infection and yields in 1923 of two lots of Goens wheat from untreated seed and seed treated by the modified hot-water method

Lot No.	Acre-yield in bushels					Percentage of loose smut	
	Seed un-treated	Seed treated					
	6 pecks per acre	6 pecks per acre		12 pecks per acre		Seed	
	Sown dry	Sown dry	Sown wet	Sown dry	Sown wet	Untreat- ed	Treated
31.....	33.2	29.2	25.5	37.1	32.2	10.4	0
35.....	27.5	29.5	26.6	40.9	34.4	11.0	0
Average.....	35.4	29.4	26.1	39.0	33.3	10.7	0

Table XVI shows the following: (1) the yield from wheat treated by the modified hot-water method was less than that from untreated wheat when both treated and untreated seed were sown at the same rate. These results confirm those obtained by Freeman and Johnson (4) in 1908 and by the writer in 1921 and 1922. (2) When the rate of seeding was increased to compensate for wheat killed in treatment, treated grain outyielded untreated grain which contained 10.7 per cent of loose smut. (3) The yield from treated seed sown wet was not so high as that from treated seed sown dry. Freeman and Johnson (4) noted that where treatment was injurious, seed improved in vitality, in general, as the length of time after treatment increased. In the foregoing experiment the dry-sown seed was treated a week before the wet-sown seed, and it seems probable, therefore, that the higher yield of the dry-sown wheat may be attributable to a better field germination. The results shown in Table XVI indicate the desirability of making a soil germination test before sowing to determine the amount of seed killed by treatment and of increasing the rate of seeding to compensate for this amount.

It has been claimed that the modified hot-water treatment kills the small shriveled kernels in a seed lot and, as a result, wheat grown from treated seed produces more vigorous plants and grain of higher bushel weight. In order to determine first the effects of treatment on large and small kernels, three lots of machine-threshed Goens and one lot of machine-threshed Leap (C. I. 4823) wheat were used. One hundred of the largest and one hundred of the smallest kernels were selected from each lot and treated on December 5, 1922. A like number were selected and left untreated. The small kernels frequently were shriveled and distorted. The treated grain was allowed to dry a week and the untreated and treated kernels were sown on December 12, in soil in greenhouse flats. One month later, on January 12, 1923, germination counts were made. The results are presented in Table XVII.

TABLE XVII.—Percentages of germination of large and small kernels of machine-threshed Goens and Leap wheat, untreated and treated by the modified hot-water method

Lot No.	Variety	Percentage of germination					
		Large kernels			Small kernels		
		Un-treated	Treated	Reduction due to treatment	Un-treated	Treated	Reduction due to treatment
32.....	Goens...	88	38	50	77	29	48
35.....	do.....	93	45	48	73	28	45
37.....	do.....	92	51	41	64	25	39
38.....	Leap....	76	23	53	66	32	34
Average.....	.....	87.3	39.3	48.0	70.0	28.5	41.5

Reference to Table XVII shows that reduction in germination due to treatment was 6.5 per cent greater for the large seeds. This probably was due to the fact that large kernels exposed more surface to threshing injury, sustained greater damage to the seed coats, and consequently suffered greater injury from treatment. Schellenberger (11) has noted that the threshing machine usually damages large kernels.

In order to determine the effects of treatment on the weight of grain, a test weight per bushel was taken of all of the wheat grown from the untreated and treated seed of five lots used in the rod-row yield experiments of 1922. The results are presented in Table XVIII.

**TABLE XVIII.**—*Bushel weight of wheat grown from five lots of untreated and of treated seed used in the yield experiments of 1922 (see Table XIV)*

Lot No.	Variety	C. I. No.	Bushel weight in pounds of wheat grown from—									
			Untreated seed sown in plats					Treated seed sown in plats				
			1	2	3	4	5	1	2	3	4	5
16	Red Chaff.....	.....	55.25	55.00	54.75	55.00	55.00	56.25	56.50	54.25	54.50	54.50
17	.....do.....	.....	54.00	56.00	52.75	53.00	53.50	54.75	57.00	53.50	53.75	53.50
19	.....do.....	.....	56.00	56.00	55.00	55.75	53.75	55.50	55.00	54.75	55.50	53.25
26	Fulcaster.....	6162	51.50	51.75	51.00	51.50	50.50	51.25	51.50	52.00	52.50	50.50
27	Purplestraw.....	1915	55.00	54.50	56.25	56.50	55.75	55.00	55.00	55.75	56.00	55.75
Average.....			54.20					54.31				

Table XVIII shows a difference in weight of grain in favor of wheat from treated seed but it amounts to less than 2 ounces per bushel.

## DISCUSSION

The effects of the modified hot-water treatment on wheat, so far as determined, have been shown to be largely dependent on the conditions of seed coats and soil. The latter varies greatly and the physical condition of the seed coats is dependent on the combination of such variable factors as the kind of weather during the period from ripening to threshing of grain, adjustment and speed of cylinder in the threshing machine, size of the wheat kernel, and handling of grain after threshing. It is evident, therefore, that the action of treatment varies and can not be predicted. The effects of treatment on the germination of machine-threshed seed were severe. The average soil germination of 58 different lots of machine-threshed seed (Tables III and VII) representing 32 distinct varieties was 33.3 per cent less for the treated than for the untreated wheat. In none of the lots did the germination of the treated seed equal that of the untreated. Even when the seed coats were intact, the modified treatment measurably retarded germination although it reduced germination very little, if at all. There is some evidence which seems to indicate that the action of treatment on spaced plants may extend beyond the germination period and into the later stages of plant growth. With regard to the effects of treatment on yield it has been shown that under the conditions of the experiments reported herein, plants from treated seed did not outyield plants from untreated seed except when sown at a rate compensating for treatment injury and when the plants from untreated seed contained a relatively high percentage of loose smut.

## SUMMARY

(1) The development in recent years of a number of central or community plants for seed-treatment in Indiana and Virginia has stimulated interest in the control of the loose smut in wheat by the modified hot-water method and in the effects of treatment on the germination, growth and yield of wheat.

(2) The method of germinating treated seed in moist blotters can not be used to determine accurately the effects of treatment on the germination of seed when sown in the soil.

(3) Other factors being equal, the effects of the modified hot-water treatment on the germination of wheat are governed by the physical condition of the seed coat.

(4) The modified hot-water treatment reduced the germination of wheat seed to zero or nearly zero when the seed coats were broken over the embryo. Seed not killed germinated abnormally.

(5) Reduction and abnormality in germination also occurred when seed coats were broken over the endosperm, but somewhat less severely. The degree of severity depended upon the location of the fracture.

(6) Seed with unbroken coats suffered a retardation in germination following treatment but there was little if any reduction in germination.

(7) Removal of the pericarp did not diminish the protective ability of an otherwise unbroken seed coat.

(8) Unbroken seed coats exerted their protective action during both the presoak and 10-minute baths of the modified hot-water treatment; the major portion during the 10-minute treatment.

(9) Increase in the duration of presoaking increased the amount of injury from the 10-minute treatment rapidly when the seed coats were broken, and relatively very slowly when the seed coats were unbroken.

(10) In the absence of any presoak period, the 10-minute treatment caused severe injury when seed coats were broken over the embryo.

(11) The rate of water absorption by the seed was more rapid when the seed coats were broken.

(12) Injury to seed coats is caused mainly in the process of threshing. All machine-threshed seed examined showed broken seed coats.

(13) Widely different weather conditions during the period between ripening and harvesting of seed had a marked effect on the amount of injury sustained by seed coats in the process of threshing and consequently in the amount of injury sustained by the seed from treatment.

(14) Small, shriveled kernels survived treatment as well or better than large, plump kernels from the same lot of machine-threshed seed.

(15) Injury and killing caused by treatment when the seed coats are broken may be due to coagulation of leucosin in the wheat embryo.

(16) Plants grown from treated seeds spaced in the rows were fewer in number and produced slightly fewer culms per plant than spaced plants from untreated seeds.

(17) Under less favorable soil conditions, the reduction in yield from treated seed was over four times the reduction in yield from untreated seed.

(18) The bushel weight of wheat grown from treated seed was not appreciably greater than that grown from untreated seed.

(19) Yield experiments were conducted for three years and a total of nine different lots was employed. In each of the three years wheat grown from untreated seed outyielded that grown from treated seed when the rate of seeding was 6 pecks per acre. In the third year, treated seed yielded better than untreated smutted seed when the former was sown dry and when the seeding rate was adjusted to compensate for wheat killed in treatment, as determined by a germination test in soil.

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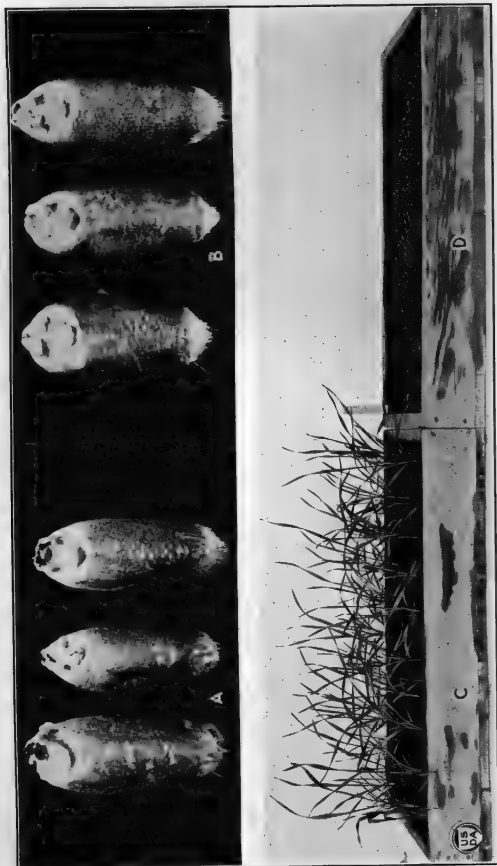
## PLATE 1

A.—Healthy head of Goens (Red Chaff) wheat in bloom and a head of the same variety affected with loose smut. The periods during which the healthy heads are in flower and spores are blowing from the diseased heads are coincident. Infection takes place intraseminally.

B.—At the close of the flowering period of the healthy heads. Practically all of the smut spores have been blown from the diseased heads.







## PLATE 2

Kernels of Goens (Red Chaff) wheat (A) and Minturki wheat (B) with seed coats broken over the embryo in threshing. (Photo.  $\times 6$ .)

Effects of the modified hot-water treatment on seeds with coats unbroken and broken over the embryo.

C.—Fifty plants from 50 hand-threshed seeds of Poole wheat, the seed coats of which were slit over the embryo with a needle, but seeds not treated.

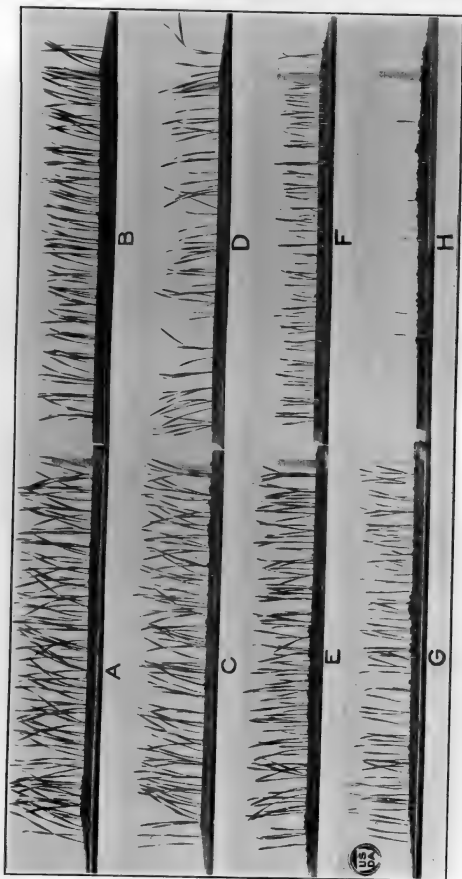
D.—Zero plants from 50 hand-threshed seeds of the same Poole wheat slit over the embryo with a needle and seeds treated.

### PLATE 3

Some of the effects of the modified hot-water treatment on seeds with coats unbroken and broken over the endosperm only.

Five seedlings from hand-threshed treated kernels of China wheat. The four distorted seedlings (right) came from seeds the coats of which had been cut over the endosperm at the brush end before treatment. The tall seedling (left) came from a treated seed with coat unbroken.





#### PLATE 4

Effects of the modified hot-water treatment on the germination and seedling development of Red May (Michigan Amber) wheat (seed lot *T* in Table VII) when threshed by hand and threshed by machine. One hundred seeds sown per flat.

A.—Hand-threshed seed, untreated. Germination 99 per cent.

B.—Hand-threshed seed, treated. Germination 100 per cent. Note retardation.

C.—Machine-threshed seed, untreated. Germination 99 per cent.

D.—Machine-threshed seed, treated. Germination 68 per cent when photographed, but final germination 77 per cent.

Effects of the modified hot-water treatment on the germination and seedling development of Dicklow wheat (seed lot *W* in Table VII) when threshed by hand and threshed by machine. One hundred seeds sown per flat.

E.—Hand-threshed seed, untreated. Germination 100 per cent.

F.—Hand-threshed seed, treated. Germination 97 per cent. Note retardation.

G.—Machine-threshed seed, untreated. Germination 84 per cent.

H.—Machine-threshed seed, treated. Germination 15 per cent when photographed, but final germination 34 per cent.

## PLATE 5

Effects of hot-water treatment on germination of hand-threshed seed of the variety Haynes Bluestem (C. I. 2874), and on seedling development, as influenced by integrity of seed coat and length of presoak. (See Table IX.)

A.—Seed coats unbroken, seed not treated. Germination 96 per cent.

B.—Seed coats cut over the embryo, seed not presoaked before treatment for 10 minutes at 54° C. Germination 38 per cent.

C.—Seed coats cut over the embryo, seed presoaked for 1 hour before treatment for 10 minutes at 54° C. Germination 19 per cent.

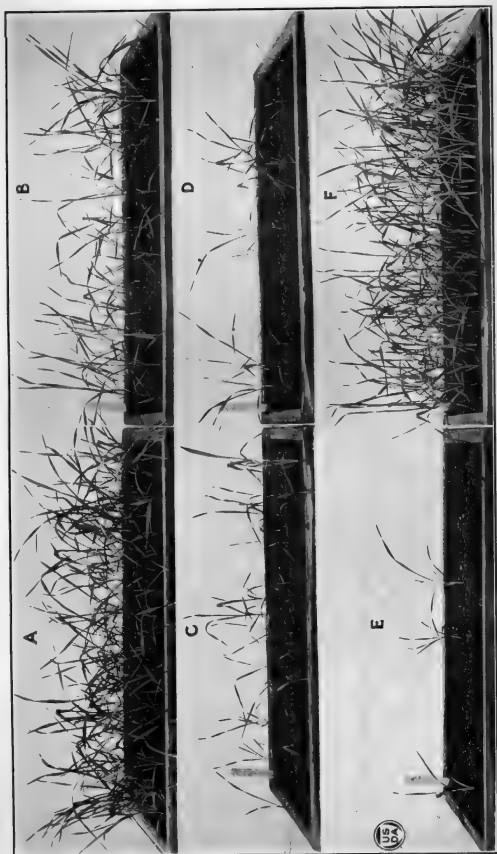
D.—Seed coats cut over the embryo, seed presoaked for 2 hours before treatment for 10 minutes at 54° C. Germination 15 per cent.

E.—Seed coats cut over the embryo, seed presoaked for 4 hours before treatment for 10 minutes at 54° C. Germination 4 per cent.

F.—Seed coats unbroken, wheat presoaked for 10.5 hours before treatment for 10 minutes at 54° C. Germination 93 per cent.

C





# JOURNAL OF AGRICULTURAL RESEARCH

## CONTENTS

	Page
Types of Vegetation in the Semiarid Portion of the United States and Their Economic Significance - - - -	99
A. E. ALDOUS and H. L. SHANTZ	
(Contribution from Department of the Interior and Bureau of Plant Industry)	
The Development of American Foulbrood in Relation to the Metabolism of Its Causative Organism - - - -	129
A. P. STURTEVANT	
(Contribution from Bureau of Entomology)	
Brittle Straw and Other Abnormalities in Rye - - - -	169
F. R. DAVISON, H. E. BREWBAKER, and N. A. THOMPSON	
(Contribution from Minnesota Agricultural Experiment Station)	
Relation between Toxicity of Cottonseed and Its Gossypol Content - - - -	173
ERICH W. SCHWARTZE and CARL L. ALSBERG	
(Contribution from Bureau of Chemistry)	
Pharmacology of Gossypol - - - - -	191
ERICH W. SCHWARTZE and CARL L. ALSBERG	
(Contribution from Bureau of Chemistry)	

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No. 2

## TYPES OF VEGETATION IN THE SEMIARID PORTION OF THE UNITED STATES AND THEIR ECONOMIC SIGNIFICANCE<sup>1</sup>

By A. E. ALDOUS, *Classifier in Charge of Homestead Classification, Geological Survey, United States Department of the Interior*, and H. L. SHANTZ, *Physiologist in Charge, Plant Geography in Its Relation to Plant Industry, Bureau of Plant Industry, United States Department of Agriculture*.

The natural or normal plant cover is a result of all growing conditions of the area where it is produced. It is therefore an index or measure of the factors influencing its growth and it serves as an indicator of the possibilities of producing other plants on the land. The differences in growing conditions have resulted in various plants and groups of plants. A number of these have been recognized as having indicator value in determining the use that can be made of the land for growing farm crops. Some plants can thrive in a wide range of growing conditions; others, however, are affected by comparatively small changes. In the various plant communities or types some species may drop out and others come in as a result of changes in growing conditions. Better or less favorable growing conditions in a region are also indicated by differences in the density of the vegetation within the same type.

In the following paper the principal types of vegetation occurring on the unreserved public lands and the patented homestead lands west of the one hundredth meridian are briefly discussed with special reference to their economic significance to crop production and grazing and some of the more important ones are illustrated. These types have been recognized and used in connection with data on topography, soil, climatic conditions, accessibility, and past agricultural history to determine the value of the lands for the production of cultivated crops and for grazing. At the time the land classification work was started there were numerous areas that had not been settled or where settlement had been so recent that no agricultural history was available. In most of these areas definite climatic data were also lacking. The vegetation therefore served as the principal means of determining the relative value of these lands for farming.

The expression "vegetation type" is here used in the sense employed by foresters to indicate a plant community of any size, rank, or stage of development. It is synonymous with the technical phytogeographic term "plant community."

In the consideration of the suitability of land for grain farming, forage, or grazing, the following 102 vegetation types have been used, which it is believed represent the most important ones to be found in the region covered by the paper. The key (pp. 117-119) summarizes the types under headings indicating their value for the production of grain or cultivated forage crops or for grazing. Owing to the fact that all the types of vegetation are used for grazing,

<sup>1</sup> Received for publication March 10, 1924.

an additional key has been prepared arranging the types according to their approximate carrying capacity. This was based on the average period of the time each year that the vegetation can be grazed without injury.

To aid in the use of the vegetation types, especially to show their range, a map was prepared dividing the western part of the United States into 15 regions, each having in a general way a similarity in vegetation. A copy of this map, together with a list of the types giving their distribution in the various regions, follows the text.

(1) GRAMA GRASS (*Bouteloua gracilis* (H. B. K.) Lag.).—The grama grass type shown by Plate 1, A, is probably the most common and abundant plant type throughout the northern and western Great Plains region. It also extends westward into the semidesert areas on the high plateaus of New Mexico, Arizona, southern Colorado, and southeastern Utah. It is composed of a relatively even stand of grama grass, presenting to the eye no other prominent plants, and grows in a rather compact fertile loam soil usually known locally as hard or tight land. The rainfall over the region occupied by this type is usually between 10 and 15 inches which penetrates the soil to a depth of 1 to 1½ feet. Areas characterized by the grama grass type of vegetation will produce good crops of wheat and other small grains only during years of more than normal rainfall. As grazing land this type is good, having a carrying capacity of from 20 to 40 head of cattle per section.

(2) GRAMA GRASS AND WHEATGRASS (*Agropyron smithii* Rydb.).—The rather heavy clay loam soils in the northern Great Plains are mainly vegetated by the grama grass and wheatgrass type, which is composed of about equal amounts of grama grass and wheatgrass. The heavier the texture of the soil, the greater the amount of wheat grass present in the type, while on the lighter soils grama grass is dominant. The heavy soil makes crop production doubtful in dry years, but relatively heavy in wet years. Considerable native hay is cut from this type. It also provides a large amount of excellent grazing, especially for cattle and horses. The number of stock that it will graze varies considerably according to the density of the vegetation but most of the lands will carry from 30 to 60 head of cattle per section.

(3) GRAMA GRASS AND NIGGERWOOL (*Carex filifolia* Nutt.).—A more or less equally mixed plant cover of grama grass and niggerwool is a very common type in the northern Great Plains. The latter species is characterized by innumerable black roots which form a dense tough sod. This type grows in well-drained loam soils, frequently where growing conditions are less favorable than for the preceding type owing to shallow or rocky soils, or to a lower precipitation. Crops can only be produced on lands supporting this vegetation during exceptionally good years. Niggerwool has a very short growing season and produces its growth early in the spring. This type is not so good grazing as Type (1), but will usually carry from 20 to 40 head of cattle per section.

(4) GRAMA GRASS, NIGGERWOOL, AND JUNEGRASS (*Koeleria cristata* (L.) Pers.).—An even mixture of grama grass and niggerwool containing an open stand of Junegrass is a common type in well-drained fertile loam soils of the northern Great Plains. A large acreage of this type has been placed under cultivation with fair success in all but the drier years. It also provides fair grazing, having a carrying capacity ranging from 30 to 60 head of cattle per square mile during the average year.

(5) GRAMA GRASS AND MOUNTAIN SAGE, also known as FRINGED WORMWOOD (*Artemisia frigida* Willd.).—This type, illustrated by Plate 1, B, is composed of a relatively pure stand of grama grass with scattered overgrowth of mountain sage. It is found in rather limited areas in the northern Great Plains occupying the higher grasslands, mesas, mountains, and valleys, as far south

as New Mexico, usually occupying smooth bench lands having a rather coarse loam soil. It is also characteristic of areas of land once plowed and revegetated, and of overgrazed areas. It characterizes land where the moisture supply is in excess of the requirements of the grama grass. This may be due to unusually heavy rainfall or to the lessened demand of the grass due to overgrazing. The latter causes the mountain sage to increase at the expense of the grama grass. As agricultural land it will produce good crops of cool-weather cereals, such as wheat and oats, except during years of less than normal rainfall. The carrying capacity of this type ranges from 25 to 50 head of cattle per section.

(6) GRAMA GRASS, NIGGERWOOL, AND SAGEBRUSH (*Artemisia tridentata* Nutt.).—Plate 2, A, illustrates this type which consists of an open stand of sagebrush growing over a comparatively even stand of grama grass and niggerwool. Frequently this type will appear as alternating small areas of relatively pure short grass and sagebrush. Common on friable loam soils in central and south-central Wyoming and northwestern Colorado. It occurs chiefly in regions where the rainfall is intermediate in character between the spring and summer type of the Great Plains and the year-round type of the Great Basin. Agriculture is very doubtful. An occasional crop may be grown during unusually good years. As grazing land it supplies considerable feed but has a low carrying capacity, ranging from 15 to 25 head of cattle per section.

(7) GRAMA GRASS AND SAGEBRUSH.—This type is usually made up of a rather thin stand of grama grass over which are growing varying densities of sagebrush ranging from a thick to a scattering stand. It also contains alternating pure patches of sagebrush and grama grass. It is very common in southwestern Colorado, southeastern Utah, and on the higher plateaus in northwestern New Mexico, in regions where the mean annual precipitation ranges from 14 to 18 inches. It may also contain varying amounts of cedar and piñon, either scattered in clumps throughout the type or bordering it on the rough lands. This type covers a rather wide range of agricultural possibilities. If, however, the sagebrush makes a thrifty rank growth the type would indicate very favorable possibilities for agriculture, including the production of small grains. The carrying capacity varies considerably, depending largely upon the amount of grama grass that is present, the sagebrush having little forage value except during the late fall and winter for sheep. The average area will graze 20 to 30 head of cattle per section.

(8) GRAMA GRASS AND CHAMISO<sup>2</sup> (*Atriplex canescens* (Pursh) James).—The grama grass and chamiso type grows in a clay soil and is widely distributed throughout the western Great Plains and plateau lands of Colorado, Arizona, New Mexico, and Utah. It is composed of a comparatively open stand of grama grass containing chamiso scattered over it at intervals of 10 to 100 feet. As grazing land it will carry from 15 to 30 head of cattle per section, but it is very doubtful if it will produce much additional feed when placed under cultivation.

(9) GRAMA GRASS AND WILD ALFALFA (*Psoralea tenuiflora* Pursh).—Plants of "wild alfalfa" (also known as scurf-pea) in this type are scattered over a sod composed principally of grama grass. It indicates a relatively abundant water supply in the deeper soil, either due to precipitation or flood water. Common on black loam soils in the northern and western Great Plains area. As agricultural land it is reasonably sure to produce a crop of wheat during years of average or more than average moisture supply. The carrying capacity of lands producing grama grass and wild alfalfa varies from 50 to 90 head of cattle per section.

(10) GRAMA GRASS AND MATCHWEED (*Gutierrezia sarothrae* (Pursh) Britten & Rusby).—Matchweed, a low, yellow-flowered shrub, 3 to 12 inches high, is scat-

<sup>2</sup> This is also known as fourwing saltbush.

tered more or less equally over grama grass in this type. Matchweed is especially abundant in rocky or shallow soils. It also comes in on the better lands when the natural vegetation is weakened by overgrazing, or continued drought, or is destroyed by plowing. This type is rather common throughout the central and southern Great Plains. The carrying capacity is low, from 5 to 15 head of cattle per section, and the land is of doubtful agricultural value.

(11) GRAMA GRASS AND VALLEY SAGE (*Artemisia nana* Nutt.).—This type, illustrated by Plate 2, B, is limited largely to the plains region of Montana, growing in a rich deep loam soil in swales and in small valleys. It consists of a scattered growth of valley sage over a grass sod composed mainly of grama grass. Agriculturally, the land will produce during years of more than average moisture supply. The grazing capacity is good, ranging from 30 to 60 head of cattle per section, the number being dependent upon the density of the vegetation.

(12) GRAMA GRASS AND WESTERN NEEDLEGRASS (*Stipa comata* Trin. & Rupr.).—A mixed stand of grama grass and needlegrass, with niggerwool, wheatgrass, and mountain sage, comprise this type. During dry seasons it appears to be composed mainly of grama grass, but during wet seasons the needlegrass is more conspicuous. By overgrazing, the mountain sage comes in and in time will become one of the dominant species of the type. This type is most important in portions of North and South Dakota and eastern Montana, on level land having a rather loose sandy black loam soil. The rainfall over the area occupied by this type ranges from 15 to 18 inches and moistens the soil to a depth of 14 to 18 inches. Agriculturally, lands of this character usually produce good crops of grains except during years of less than average moisture supply. They also afford very good grazing, ranging from 40 to 80 head of cattle per section in most areas. The western needlegrass, however, is not readily grazed after it matures, owing to its tough texture.

(13) GRAMA-BUFFALO GRASS (*Bulbilis dactyloides* (Nutt.) Raf.).—The grama-buffalo grass type, shown by Plate 3, A, which is composed of a mixture of grama and buffalo grasses, is one of the most extensive types on the Great Plains south of the Montana line. It grows on hard or tight land where the soil moisture only rarely penetrates below the second foot and crop failures are sure during years of less than average moisture supply. Corn and wheat are the chief crops in the North, while the grain sorghums are ever present in the South. The carrying capacity over most of the lands supporting this type ranges from 25 to 50 head of cattle per section.

(14) GRAMA-BUFFALO GRASS, AND WESTERN NEEDLEGRASS.—A mixed stand of grama grass, buffalo grass, and needlegrass makes up this type. The needlegrass is taller than the other grasses and is more conspicuous, especially in wet years. The type also usually contains a scattering of Psoralea (*P. argophylla*), cone flower (*Echinacea*), and other flowering plants. Its soil and range are similar to Type (13). It is productive for wheat and corn in the North and grain sorghums in the South during years of average or more than average moisture supply. The carrying capacity of this type ranges from 35 to 75 head of cattle per section.

(15) GRAMA-BUFFALO, AND WIRE NEEDLEGRASS (*Aristida longiseta* Steud.).—This type, illustrated by Plate 3, B, is composed of an even cover of grama and buffalo grass, with scattered bunches of wire needlegrass. This is a common type on loose, sandy loam soils throughout the western Great Plains region. On heavy land this type occurs on the eastern edge of the high plains. Agriculturally, this land is productive of wheat, corn, and grain sorghums during years of average or more than average moisture supply. As grazing land, it will carry from 20 to 40 head of cattle per square mile. The wire needlegrass is too tough to be palatable when it matures.

(16) GRAMA-BUFFALO, AND WHEATGRASS.—Large areas of rather heavy soils, mostly clays and clay loams throughout the Great Plains region, especially

in western South Dakota, eastern Wyoming, and western Nebraska, are vegetated by this type. It consists of a sod of grama grass and buffalo grass and varying amounts of wheatgrass. In dry years the short grasses are more abundant, but in wet years the wheatgrass appears to be the dominant species. Considerable native hay is cut from lands containing this type. It also furnishes a large amount of grazing for range stock. The carrying capacity varies from 30 to 75 head of stock per section. The heavy soil makes crop production doubtful during dry years, but relatively heavy during years of excessive rainfall.

(17) GRAMA-BUFFALO, AND WILD ALFALFA.—The vegetation of this type is composed of an even sod of grama grass and buffalo grass over which plants of wild alfalfa are more or less equally scattered. The soil is similar to Type (9). This type does not occur to any appreciable extent north of Yellowstone River in Montana or in North Dakota. It indicates more than usual water supply, due either to precipitation or to flood water. The soil is deep as compared with Type (13), and it will often produce crops when the pure short grass land fails. The carrying capacity on most of the lands producing this type ranges from 30 to 65 head of cattle per section.

(18) GRAMA-BUFFALO GRASS, AND SOAPWEED YUCCA (*Yucca glauca* Nutt.).—The vegetative covering of this type is composed of an even stand of grama-buffalo, the latter usually predominating, with scattering bunches of soapweed yucca. It is very common in the southern Great Plains, growing on a loose, sandy loam soil. Land producing this type of vegetation is successfully used for growing corn and grain sorghums, and in some of the more favorable localities good yields of wheat are obtained in years of more than average precipitation. It provides nutritious and palatable forage, having a grazing capacity of 25 to 50 head of cattle per square mile.

(19) GRAMA-BUFFALO, AND MESQUITE (*Prosopis glandulosa* Torr.).—This is a very common type in the southern Great Plains of western Texas and eastern New Mexico, growing on hard lands. It is composed of an open sod of grama grass and buffalo grass, over which are scattering bunches of mesquite. Rainfall varies from 15 to 18 inches over the region occupied by this type. Moisture is insufficient, except in years of abnormal rainfall, to produce any cultivated crops other than sorghums. This type has a carrying capacity of 40 to 60 head of cattle per section.

(20) GRAMA-BUFFALO GRASS, AND MATCHWEED.—Plate 4, A, shows this type, which is made up of a sod of grama grass and buffalo grass over which is scattered matchweed in varying densities. The distribution is the same as Type (13). Where it occurs naturally it indicates land of doubtful agricultural value and grazing land of low carrying capacity, varying from 15 to 25 head of cattle per square mile. This type develops on land previously characterized by Type (13) as a result of overgrazing or breaking.

(21) WHEATGRASS.—This grass usually does not form a sod, but is found in open stands consisting of upright single plants scattered uniformly over the surface. It is widely distributed throughout the western United States, growing in heavy clay soils that usually contain alkali. It is very common in southeastern Montana and northwestern South Dakota, and it is of doubtful agricultural value, due mainly to the heavy soils in which it grows. In years of abnormal precipitation, however, high yields of grain have been obtained on lands producing this type. It is very palatable and nutritious to all classes of livestock, but because of the open stand and tendency to fail during dry years its carrying capacity ranges as low as 10 head of cattle per section. The better areas will support 50 head per square mile.

(22) WIRE NEEDLEGRASS.—In addition to wire needlegrass, this type frequently contains patches of short grass and a scattering of flowering plants. It is a common



type in the Great Plains, growing in a rather loose sandy or gravelly loam soil. Toward the eastern side of the Great Plains it grows on a heavy soil. As agricultural land it is equal to, or possibly superior to, Type (15), which it resembles closely. Wire needlegrass has very little grazing value and is only taken by stock when other feed is scarce. However, the short grasses produced in the type afford fair grazing. The poorer gravelly lands producing this type have a low carrying capacity of not more than 15 head of cattle per section. The better lands, however, will graze double this number.

(23) NIGGERWOOL.—This type which is composed of a more or less pure stand of niggerwool occupies rather a limited area in the Great Plains section of Wyoming and Montana, growing usually on ridges or benches, where growing conditions are less favorable, due to shallow rocky soil or low rainfall. The land is of very little value for producing crops unless water is available for irrigation. Niggerwool is rather tough, and therefore has a low carrying capacity, 5 to 15 head of cattle per section.

(24) WESTERN NEEDLEGRASS.—Although needlegrass is widely distributed throughout the western United States, this type, which is composed of a medium stand of needlegrass containing scarcely any other plant species, has only been observed to any appreciable extent in northeastern Montana and northwestern North Dakota, where it occupies smooth to gently rolling bench lands, having a deep fertile black loam soil. This land is very fertile and easily tilled and since it occupies areas where the rainfall ranges from 15 to 18 inches, good crops of small grain can be produced in all but the dry years. This species affords fair forage when young but when it matures is too tough to be readily grazed by stock. Most of the tillable better lands that support this type are being successfully used for producing crops, mainly wheat.

(25) MATCHWEED.—This low-growing suffrutescent plant seldom occurs in pure stands except over burned areas or areas that have been greatly overgrazed or on old breakings. Its predominance on these locations is only temporary as it is gradually replaced, on the return to normal conditions, by the species originally growing on these lands. It is native, however, to rocky, gravelly areas, or on lands having very shallow soil where it is generally associated with a few other species. This type is very common in the southern Great Plains and in the Great Basin. The acreage occupied by its natural habitat is rather small, however, regardless of its common occurrence. It is worthless for grazing, which accounts partly for its invasion of overgrazed areas. In its natural habitat it indicates nonagricultural land unless irrigated.

(26) MATCHWEED AND MOUNTAIN SAGE (*Artemisia frigida* Willd.).—The matchweed and mountain sage type is found throughout the Great Plains on smooth bench lands having a rather coarse or gravelly loam soil. In the northern part of the Plains, mountain sage is more important and matchweed in the southern part. In the central Plain region this type indicates conditions intermediate between Type (25) in the South and Type (5) in the North. It consists of a mixed stand of matchweed and mountain sage growing over an open stand of short grass (grama or grama-buffalo), and characterizes old breakings, overgrazed land, and shallow soils where conditions are not favorable for a short grass cover. The type has low value for grazing.

(27) BLACK GRAMA (*Bouteloua eriopoda* Torr.).—The black grama type is composed of a relatively pure cover of black grama with scarcely any other species. It does not form a sod cover but branches out loosely to produce a relatively large amount of top growth. This species is dominant on large areas of grassland in the southwestern part of New Mexico and southeastern Arizona, where it grows in a light loam soil. It is of little value for growing crops except in good years when forage crops can be successfully produced. It is a very important range

grass, being highly palatable to all livestock. Carrying capacity ranges from 15 to 30 head of cattle per section, and in very favorable years, it makes sufficient growth on very favorable habitats to be cut for hay.

(28) **HAIRY GRAMA** (*Bouteloua hirsuta* (Lag.)), **GRAMA**, and **SAND SAGE** (*Artemisia filifolia* Torr.).—This type grows on sandy soils throughout the central Great Plains region, especially eastern Colorado, and is made up of an open stand of sand sage growing over an even sod composed mainly of hairy grama and grama. It has doubtful agricultural value except for sorghum and corn, since the soil is likely to blow if cultivated. As grazing land it will carry from 15 to 25 head of cattle per section. It will not stand heavy grazing owing to the loose texture of the soil. The sand sage also comes in as a result of overgrazing which decreases the grazing capacity of the range.

(29) **BUNCHGRASS** (*Andropogon scoparius* Michx.).—Lands on which an open stand of bunchgrass comprises most of the vegetation are classified under this type. It occurs on the sandhills and rough, rocky, or gravelly hills of the central or western high plains. It should not be confused with the relatively pure close stands of this grass on the loam soils of the eastern and southern high plains. The best type of this land is good corn or sorghum land, but in the western portion it often characterizes nonagricultural land. Bunchgrass when young is very palatable and nutritious to livestock and is also valued as winter range since it is not so easily covered with snow as the short grasses.

(30) **SAND HILLS MIXED**.—Plate 4, B, illustrates a typical sand hills mixed type which consists of a mixture or alternation of sand sage, bunchgrass, big blue stem (*Andropogon furcatus* Muhl. or *Andropogon hallii* Hack.), sandgrass (*Calamovilfa longifolia* (Hook.) Scribn.), hairy grama (*Bouteloua hirsuta* Lag.), and frequently a number of flowering plants. It occurs on sand hills or rolling sandy land throughout the central and southern Great Plains, but is probably most common in the sand hills of eastern Colorado. This type of range supplies considerable forage and is especially valuable in dry years when little feed is produced on the hard lands. Its carrying capacity is from 20 to 50 head of cattle per section. As agricultural land it is subject to blowing and the whole area may be transformed by breaking into a blowout.

(31) **BLACK GRAMA AND WIRE NEEDLEGRASS**.—Lands having a uniform sod of black grama with varying quantities of wire needlegrass are listed under this type. The amount of wire needlegrass present in this type is usually governed by soil conditions, this species being more abundant on lands having coarse gravelly soils. A very common type in southwestern New Mexico and eastern Arizona, growing on slightly gravelly, rocky, or sandy loam soils. Areas producing this type can be used for growing forage crops only in favorable seasons. Black grama is excellent forage, but wire needlegrass is grazed only for a short time after growth starts. The carrying capacity of the black grama and wire needlegrass type ranges from 15 to 30 head of cattle per section.

(32) **BLACK GRAMA AND TOBOSA** (*Hilaria mutica* (Buckl.) Benth.).—This type is made up of varying quantities of tobosa grass growing in a relatively even stand of grass composed mainly of black grama. It usually grows in rather fine compact soils on flats that receive some flood water. The amount of tobosa grass in this type is directly proportionate to the amount of flood water received. This vegetative type occupies quite a large acreage in southwestern New Mexico and southeastern Arizona and possesses a farming and grazing value similar to Type (31).

(33) **TOBOSA GRASS**.—The tobosa grass type is applied to vegetative associations that are composed almost entirely of tobosa grass. This is the predominant type on flats and swales in New Mexico and eastern Arizona that are subject to flooding, where the soil is a fine compact loam or a clay loam. It occupies land

too arid to use for crop production unless additional moisture is available from flooding. The kind and amount of crops raised would depend upon the quantity of flood water. Tobosa grass is grazed when green but is practically worthless when dry.

(34) *GALLETA* (*Hilaria jamesii* (Torr.) Benth.).—Galleta grass is found in pure stands in this type. It is usually grazed rather closely so that it forms an even turf. This species is common in New Mexico and eastern Arizona but it occupies rather small areas. In Utah and Nevada, however, it occurs over rather large areas and it constitutes one of the best grazing types in the Great Basin. Very good grazing for all classes of livestock and can withstand heavy grazing. Usually more valuable for the native forage it provides than for growing cultivated crops. The carrying capacity varies considerably according to the density of the galleta. On most areas, however, the grazing capacity will range from 25 to 100 head of cattle per section.

(35) *CROWFOOT GRAMA* (*Bouteloua rothrockii* Vasey).—This species while found in combination with other species often forms pure stand and during wet years it makes sufficient growth on the more favorable habitats to cut for hay. Common in Arizona on mountain mesas and mountain foothills where it occupies a deep black loam soil. The most favorable habitats of this type can be used for producing cultivated forage crops. Crowfoot grama is highly palatable and nutritious but will not stand heavy grazing. It has a carrying capacity of from 20 to 35 head of cattle per section.

(36) *Hilaria rigida* Benth.—This grass resembles a shrub in its coarse upright growth. Its range is limited largely to the Colorado desert where it is frequently found in pure stands of rather scattered bunches. It develops best in areas where sand is slowly being deposited over the soil. It is almost too coarse to be readily taken by stock but is one of the main perennial grazing plants in the desert regions, so that it affords considerable feed. The habitat of this species is too arid to produce any cultivated crop without irrigation. It has a carrying capacity of from 2 to 10 head of cattle per section.

(37) *TUSsock GRASS* (*Sporobolus airoides* Torr.).—Tussock grass, illustrated in Plate 5, A, is a rather coarse-tufted grass forming a big bunched growth often a foot in diameter at the base. It is common along water courses on rather heavy alkaline or "gyp" soils in the southern Great Basin, New Mexico, and Arizona, and adjacent portions of the High Plains. Readily grazed by cattle and horses when green. It is most abundant on alkali land that receives some flood water. Lands occupied by this type usually contain too much alkali to be used for growing crops. It affords the best grazing of any of the alkali grasses, having a carrying capacity of from 50 to 100 head of cattle per section.

(38) *SACATON* (*Sporobolus wrightii* Munro).—Sacaton grows in tall coarse bunches forming a dense bunchlike stand on deep alluvial land relatively free from harmful amounts of alkali. It therefore characterizes land excellent for irrigation. It is found in eastern Arizona, New Mexico, and western Texas. Sacaton makes good hay, especially for horses. It is fairly good for grazing but is easily killed out by excessive tramping. It has a carrying capacity of from 50 to 100 head of cattle per section.

(39) *WHEAT BUNCHGRASS* (*Agropyron spicatum* (Pursh) Rydb.).—The wheat bunchgrass type illustrated by Plate 5, B, is applied to open grass lands composed almost entirely of wheat bunchgrass. This is the dominant type in the Columbia River Basin in Washington and below the timber zone in northeastern and north-central Oregon. It is also common in the rolling foothills and lower plateaus of Idaho and northern Utah, growing on well-drained loamy soils. Most of the best wheat lands of Oregon and Washington were originally vegetated by this type, so where it is growing in dense thrifty stands it is indicative of

favorable possibilities for grain production. It affords very good grazing, especially for cattle and horses, and has a carrying capacity ranging from 25 to 60 head of cattle per section.

(40) **CURLY BUNCHGRASS** (*Festuca ovina* L.).—Curly bunchgrass is a very common type on rolling foothills of eastern Idaho, western Colorado, and northern Utah, growing in deep rather coarse loam soil. It is frequently associated with Junegrass (*Koeleria cristata* (L.) Pers.), blue grasses (*Poa*), and *Stipa*. It frequently merges into the sage-brush type, where it is the dominant herbaceous species. The moisture requirements of this type are such that the lands it occupies can be successfully used for the production of wheat and oats, the main limitation being the length of growing season, which is frequently too short to mature grains. It affords excellent grazing, especially for cattle and horses, having a carrying capacity of from 30 to 60 head of cattle per section.

(41) **MIXED MOUNTAIN GRASS**.—A mixed stand of grasses composed mainly of bluegrass (*Poa*), curly bunchgrass, Junegrass, wheat bunchgrass, and needlegrass (*Stipa*), make up this type. About one-third of the stand of vegetation may also be composed of weeds, principally wild geranium (*Geranium incisum* Nutt.), alum root (*Sieversia ciliata* (Pursh) G. Don.), balsam root (*Balsamorhiza sagittata* (Pursh) Nutt.), lupines, sunflower (*Helianthella* species), and Senecios. This type is common on rolling foothills and lower mountain lands of Colorado, Utah, and Idaho, usually occupying well-drained deep loam soils. The growing season is usually too short to mature cultivated crops, but this type furnishes excellent grazing, having a carrying capacity that ranges from 50 to 100 head of cattle per section for a four to five months grazing season.

(42) **ARIZONA FESCUE** (*Festuca arizonica* Vasey).—Grass lands where *Festuca arizonica* is the predominant species are classified in this type. Its range is confined to foothill and mountain areas above 6,000 feet in Arizona and New Mexico. It is the most common mountain bunchgrass in the Southwest, and is frequently the predominant herbaceous species in open stands of yellow pine timber. As agricultural land it is capable of crop production without irrigation during exceptionally good years. The growth season is best adapted to small grains. It affords excellent forage during its early season of growth, but on maturity it becomes too coarse to be grazed. It has a carrying capacity which ranges from 20 to 30 head of stock per section.

(43) **SHORT-LIVED GRASS TYPE**.—This type consists of a mixture of small short-lived grasses. The following are often prominent: *Scleropogon brevifolius* Phil., *Aristida bromoides* H. B. K., *Bouteloua aristoides* (H. B. K.) Griseb., and short-season annuals. It is widely distributed in the Southwest, growing on mesas and foothills where desert or semidesert conditions prevail in a loamy soil, sometimes slightly sandy. This type usually marks badly overgrazed areas and is not therefore indicative of the character of the soil or potential grazing value.

(44) **GIANT WILD-RYE** (*Elymus condensatus* Presl).—This species, illustrated in Plate 6, A, grows in a deep rich alluvial soil free of harmful quantities of alkali. It is also common in waste places, such as along ditch banks. It is widely distributed throughout the Northwestern States, found usually in small patches and in isolated bunches, and is very conspicuous due to its tall bunchy growth, but the area it occupies is rather small. It marks land unusually valuable when placed under irrigation and is of very little grazing value owing to its coarse texture.

(45) **WEED GRASS**.—This type is limited chiefly to California where it is the principal type on the valley floors and lower foothills on lands containing little or no alkali. It consists of a more or less even stand of annuals or short-lived grasses, chief among which are three species of brome grass (*Bromus* species), wild oats (*Avena fatua* L.), and filare, also known as alfilaria, (*Erodium cicutarium* (L.) L'Hér.). In Washington and Oregon a similar type occurs on overgrazed

lands. It indicates lands from which the original vegetation has been driven out by overgrazing. In California this was done during early Spanish days. Although it has little indicative significance from the standpoint of crop production, much of the land characterized by this type is capable of producing crops of wheat and other small grains without irrigation. It supplies practically all the feed in the unimproved pastures and ranges in the valleys and lower foothills of California. The carrying capacity is from 15 to 30 head of cattle per section.

(46) WET MEADOW.—Classified in this type are all areas that remain wet or moist throughout the summer, where sedges and rushes are the dominant species of vegetation. These lands are usually located in the mountain valleys and are quite generally used for cutting wild hay. They have a rich black peaty soil, which, if drained, makes very good farm lands, but they are usually located at elevations too high for grains to mature. These lands afford very good grazing, carrying capacity ranging from 100 to 300 head of cattle per section, and are located throughout the mountainous areas of Colorado, Utah, and the Northwest.

(47) DRY MEADOW.—Open parks that are wet during the early part of the summer, but become moderately dry during the latter part of the season are included in this type. It includes the "Camas Prairies," so common in Idaho, and is composed of a mixture of weeds and grasses. The weeds are confined mainly to common camas (*Quamasia quamash* (Pursh) Coville), white flowered dock (*Polygonum*, *Wyethia* sp.), while the grasses are principally red top and other species of *Agrostis*. The growing season is usually too short for maturing cultivated farm crops. However, it supplies good grazing, having a carrying capacity ranging from 50 to 100 head of cattle per section. Its range is Colorado and the Northwest.

(48) MOUNTAIN WEED.—This type includes all untimbered mountain lands where nongrasslike species of herbaceous vegetation predominate. The principal species found in this type are wild geraniums, lupines, *Senecio*, white clover, dandelions, tall larkspurs, umbellifers, and *Potentillas*. This type is very common in moist rich soils in the higher mountainous areas in Colorado and the Northwest. It has not been utilized for crop production, mainly on account of the short growing season, but provides excellent summer range for sheep, having a carrying capacity of from 300 to 600 head of sheep per section.

(49) SAGEBRUSH (*Artemisia tridentata* Nutt.).—The sagebrush type illustrated by Plate 6, B, consists of a miniature forest or scattered brush land of sagebrush. Plants vary in height from 1 to 7 feet. This is the most common shrub throughout the Great Basin, growing in well-drained loamy soils. The height and abundance of plants are governed by the depth, quality, and moisture content of the soil. A dense stand of tall sagebrush is indicative of very favorable possibilities for the production of small grains without irrigation. It represents the best type of land in the Great Basin for either arid or irrigated farming. This type is grazed by stock, mainly sheep, during fall and winter when little or no herbaceous feed is available.

(50) SMALL SAGEBRUSH (*Artemisia nova* A. Nels.).—Plate 7, A, illustrates this type, which is a lower growing sagebrush than the preceding species, ranging in height from 1 to 3 feet and presenting during the growing season a more yellowish color than the sagebrush. The range of this plant is similar to that of sagebrush, but it occupies a rather limited area on rocky or shallow soils. As agricultural land this type is far inferior to a cover of pure sagebrush. The grazing value is rather low, 5 to 10 head of cattle per section.

(51) SCAB SAGEBRUSH (*Artemisia rigida* (Nutt.) A. Gray).—Lands containing a comparatively even stand of scab sagebrush, a species ranging from 1 to 2 feet

high, having twisted gnarled stems, and a comparatively small amount of foliage, are classified in this type. It occurs principally on the scablands in Washington and Oregon, growing on rocky soil, wholly unsuited for crop production. The grazing value is poor, less than 10 head of cattle per section.

(52) **HOP-SAGE** (*Grayia spinosa* (Hook.) Moq.).—Hop-sage resembles sagebrush when not in leaf, but has thick fleshy leaves which contrast sharply with the thin silvery leaves of the sagebrush. It is called "hop-sage" because of the character of the fruits which resemble hops. This species is very abundant and has a wide range throughout the very arid sections of the West, particularly Nevada and western Utah. It is also found in the Red Desert of Wyoming, growing with shadscale and sagebrush. Land characterized by this type is of little or no value for crop production unless irrigated. It is of little forage value except as winter range for sheep.

(53) **ANTELOPE-BRUSH** (*Purshia tridentata* (Pursh) DC.).—The antelope-brush type, shown by Plate 7, B, is dominant in sandy soils along the Columbia River in Washington and Oregon. It also occurs through the Great Basin region as well as in California. As a rule it is associated with sagebrush. Together they usually characterize land more rocky and therefore less suited to crop production than pure sagebrush. They supply a fair browse for cattle and sheep, having a carrying capacity of 20 to 30 head of cattle per section.

(54) **SHADSCALE** (*Atriplex confertifolia* (Torr.) S. Wats.).—Plate 8, A, illustrates a typical shadscale area which supports scattered even stands of shadscale, the plants ranging from 6 inches to 2 feet in height and usually presenting a uniform rounded appearance. The leaves are leathery and seeds are produced in abundance. Plants are spiny and too rigid to be moved by an ordinary wind. This is a very common and widely distributed type in the Great Basin, growing frequently in rather heavy alkaline soils. In most localities it characterizes a shallow soil with alkali in the second foot and also to a lesser extent in the first foot. It is nonagricultural unless irrigated and drained to remove excess alkaline salts. It is one of the main plants grazed by sheep on the winter ranges. It has a carrying capacity of 25 to 100 head of sheep per section for a short intermittent grazing period.

(55) **SALT SAGE** (*Atriplex corrugata* S. Wats. and *A. nuttallii* S. Wats.).—This species, also known as saltbush, illustrated by Plate 8, B, grows in low mats varying in size from a few inches to several feet across and seldom exceeding 6 inches in height. The mats are often scattered over an otherwise barren surface. It is found in rather heavy saline soils in southwestern Wyoming, west-central Colorado, east-central and western Utah, and Nevada. Readily browsed by sheep during the winter season. Growth occurs very early in the season, and it is then readily grazed by sheep. It has a carrying capacity of 25 to 75 head of sheep per section. This species indicates nonagricultural land, owing mainly to the high alkali content of the soil.

(56) **LITTLE RABBITBRUSH** (*Chrysothamnus stenophyllus* (A. Gray) Greene).—This type, illustrated by Plate 9, A, is applied to lands containing a rather even growth of small bunches, or of scattered larger bunches, of little rabbitbrush. Flowers are produced in great quantity and the area presents a uniform bright yellow color during the flowering period. The bushes are often as much as 2 feet high, but usually much smaller. It is indigenous to Utah and Nevada on light soils free from alkali. This species very frequently comes in on burned sagebrush and shadscale land. It has little value as grazing land.

(57) **RABBITBRUSH** (*Chrysothamnus graveolens* (Nutt.) Greene), and **TUSsock GRASS**.—Lands containing a fairly even or occasionally matlike sod of tussock grass over which are scattered plants of rabbitbrush are classified in this type. Areas supporting this type have too much alkali for growing cultivated crops.

As a rule, part of its moisture supply comes from flood water. It is good grazing land but nonagricultural, except with drainage and careful irrigation. It has a carrying capacity of 20 to 40 head of cattle per section.

(58) WINTER-FAT (*Eurotia lanata* (Pursh.) Moq.).—A representative winter-fat type is shown on Plate 9, B, consisting of a scattering growth of winter-fat with little or no other species of vegetation. The winter-fat plants are very white and hairy and range in height from 3 to 12 inches and occasionally more. On account of its white appearance it is sometimes called white sage. This species grows on rather heavy soils in the more arid sections of the West, where it occupies rather a large area. It indicates land unsuitable for agriculture unless irrigated. Winter-fat is especially palatable and nutritious to sheep and supplies considerable forage on the winter ranges. In the Great Basin it is probably the best grazing land to be found in the desert regions.

(59) WHITE SAGE, also known as GREEN MOLLY (*Kochia americana* S. Wats.).—Lands are classified in this type which contain an open to scattering growth of white sage, a low plant, seldom exceeding 6 inches in height, having hairy round leaves that are more fleshy than those of winter-fat. It resembles winter-fat in general appearance, although it is not grazed to any appreciable extent. This type is not widely distributed throughout western Utah and Nevada, growing in heavy clay-loam soils. It is of doubtful agricultural value and of little grazing value.

(60) COLEOGYNE (*Coleogyne ramosissima* Torr.).—This type, illustrated by Plate 10, A, consists of an even growth of Coleogyne and a scattering of cliff-rose (*Cowania stansburiana* Torr), shrub buckwheat (*Eriogonum fasciculatum*), and tomatilla (*Lycium pallidum* (Miers). A very common type in the desert area of northwestern Arizona, southeastern California, southwestern Utah, and southern Nevada, where it occupies a region between that characterized by creosotebush and sagebrush. It usually grows in dry, porous desert soils. The land is of no value for crop production unless irrigated, when it is very valuable owing to the long growing season of the region it occupies. Coleogyne has no value for forage, so the type has a very low carrying capacity for livestock.

(61) GREASEWOOD (*Sarcobatus vermiculatus* (Hook.) Torr.).—This species, shown by Plate 10, B, is limited to subirrigated alkali lands throughout the West. The leaves of this plant are fleshy and the stems woody and spiny, ranging in height from 1 to 5 feet. The leaves are bright green in color and have a salty taste. This plant is a good indicator of lands having a rather heavy concentration of alkali. Under the proper system of irrigation land of this type will produce good crops, but unless drainage is supplied it is likely to develop excess quantities of alkali. The young branches of greasewood are readily grazed by sheep and cattle, especially during the winter.

(62) GREASEWOOD-SHADSCALE.—The greasewood-shadscale type consists of an even scattered growth of shadscale with an occasional scattering of plants of greasewood. It occurs on rather heavy alkaline soils in the Great Basin region, especially in Utah and Nevada. Under irrigation it can become productive if drained, but without irrigation it is worthless for crop production. It is relatively poor grazing land, mainly valuable as winter range for sheep, with a carrying capacity of 25 to 75 head of sheep per section.

(63) GREASEWOOD, SALT SAGE.—This type is commonly found in comparatively heavy alkali lands throughout western Wyoming and the Great Basin. It is composed of a very even scattered growth of salt sage over which at rather wide intervals occur large plants of greasewood. Its agricultural possibilities are similar to Type (55) but it has a larger amount of moisture penetration or possibly subsoil moisture. The grazing value is similar to the preceding type.

(64) GREASEWOOD, SALTGRASS (*Distichlis spicata* (L.) Greene).—Areas containing scattered plants of greasewood on a sod formed by saltgrass are classified

in this type. Saltgrass usually has a salty taste and is low-growing, seldom exceeding 8 inches in height. A very common type on alkali lands in the Great Basin region. Owing to excessive amounts of alkali found in the soil, it is non-agricultural. The type affords relatively good grazing land for cattle and horses, however. The carrying capacity is dependent upon the density of the saltgrass; 10 to 40 head of cattle per section.

(65) **SALTGRASS**.—This type, illustrated by Plate 11, A, occupies a large area of land in the Great Basin and even in portions of the Great Plains that have a shallow water table and contain a large alkali content. It may also contain barren alkali spots, the percentage of the surface occupied by these spots being governed by the concentration of alkali in the soil. Lands producing this type are nonagricultural unless the excess alkali is drained out. Palatable to cattle and horses until it matures when it is too tough for forage. It will stand very close grazing without injury and has a carrying capacity of 50 to 150 head of cattle per section.

(66) **PICKLEWEED**<sup>3</sup> (*Allenrolfea occidentalis* (S. Wats.) Kuntze).—The pickleweed type illustrated by Plate 11, B, contains a scattered growth of small plants or widely spaced larger plants of pickleweed. While the basal portion of this plant may be large and woody, the upper stems are thick and juicy and easily crushed in the hand. The fleshy stems are about one-eighth of an inch in diameter. Pickleweed grows on very alkaline soils in the Great Basin region. It has no grazing value and the land it occupies is nonagricultural.

(67) **SEEPWEED** (*Dondia moquinii* (Torr.) A. Nels.).—Seepweed is a common plant in the Great Basin, Colorado Desert, and adjacent valleys. It usually grows in rather dense pure stands in heavy alkaline soils. The branches of seepweed are very small and delicate and the leaves fleshy and easily crushed in the hand. The flowering branches often appear dark near the ends. It is worthless for agriculture and grazing.

(68) **SAMPHIRE** (*Salicornia utahensis* Tidestrom and *Salicornia rubra* A. Nels.).—This type occurs only under extremely saline conditions. The plants are very fleshy, seldom exceeding 6 inches in height, and have a very salty taste. It can be distinguished from pickleweed by the opposite leaves and branches. There are two types, the annual form (*Salicornia rubra*) and the perennial form (*Salicornia utahensis*). The former is usually much branched, the latter having as a rule only one or two branches. It grows in rather restricted areas throughout the Great Basin. It is of no value for grazing and characterizes nonagricultural land.

(69) **CREOSOTEBUSH** (*Covillea glutinosa* (Englem) Rydb.).—Plate 12, A, shows an average tract of land vegetated by the creosotebush type, which consists of widely spaced plants of creosotebush growing either on barren soil or soil covered by winter or summer annuals, such as plantain, etc. This plant is often locally known as greasewood and is the most common shrub in the desert sections of southeastern California, southern Nevada, and western Arizona, where it grows in gravelly well-drained soil. Farther east where rainfall is more abundant it occurs on very shallow soils, soils with caliche near the surface. This is especially true in southeastern Arizona, southern New Mexico, and southwestern Texas. It is nonagricultural unless irrigated, and very poor for grazing, having a carrying capacity of less than 10 head of cattle per section.

(70) **CREOSOTEBUSH AND BUR-SAGE** (*Franseria deltoidea* Torr., *F. dumosa* A. Gray).—The creosotebush and bur-sage type, illustrated by Plate 12, B, is composed of an open to scattering stand of creosotebush with about an equal amount of two species of bur-sage. This type also frequently contains a scattering of paloverde, catsclaw, ironwood, mesquite, and giant cactus (*Carnegiea gigantea* (Engelm.)). It is dominant in broad valleys of western Arizona

<sup>3</sup> This is also known as burro-weed.



and southeastern California, where the mean annual rainfall is less than 10 inches. This type is nonagricultural unless irrigated. Neither creosote bush nor bur-sage has any grazing value. In wet years, however, annuals appear on the range occupied by this type which afford a limited amount of feed for stock. It has a carrying capacity of 0 to 10 head of cattle per section.

(71) **ENCELIA** (*Encelia farinosa* A. Gray) and **CALIFORNIA SAGEBRUSH** (*Artemisia californica* Less.).—This is a very common type in the dry inland valleys of California. It consists of a shrubby growth of *Encelia farinosa*, a rather coarse-stemmed plant with sunflowerlike flowers bearing rigid, white, and light-colored leaves; and *Artemisia californica*, a plant of similar size with very finely divided leaves and darker appearance. These shrubs have no value for grazing and the lands they occupy have no value for crop production, unless irrigated.

(72) **SHRUB BUCKWHEAT** (*Eriogonum fasciculatum* Benth.).—Shrub buckwheat is the dominant species in this type. It also contains scattering plants of Joshua tree (*Yucca arborescens*). With this type are often mixed a large number of shrub plants and occasionally juniper and creosotebush. This type is found in the desert regions of southeastern California and southern Nevada; its agricultural and grazing value are similar to the preceding type.

(73) **DESERT BRUSH TYPE: CREOSOTEBUSH, IRONWOOD** (*Olneya tesota* A. Gray), **MESQUITE, PALOVERDE** (*Parkinsonia microphylla* Torr.), and **CATSCRAW** (*Acacia greggii* A. Gray).—This is a mixed desert brush type in which creosotebush, mesquite, paloverde, ironwood, and catsclaw are the five important species. It is common in the desert region occupying large portions of western Arizona and southeastern California. It usually occurs along drainage channels. The aridity of the region where this type is found precludes the possibility of producing crops without irrigation. Mesquite and paloverde are the only species that have any appreciable grazing value. It has a carrying capacity of less than 10 head of cattle per section.

(74) **CREOSOTEBUSH-DESERT GRASS**.—A typical creosotebush-desert grass area is illustrated by Plate 13, A. It is composed of a scattered growth of creosotebush over grass land consisting usually of *Bouteloua rothrockii*, *Bouteloua eriopoda*, and *Hilaria mutica*. This type grows on loamy soils in southern New Mexico and Arizona. With the exception of *Hilaria mutica*, all the grasses growing in this type are highly palatable. It has a carrying capacity of 10 to 20 head of cattle per section. The land producing these species is too arid to grow crops unless irrigated.

(75) **CREOSOTEBUSH AND CACTI** (*Opuntia* species).—This type grows in rather broken or rocky areas in southern New Mexico and the desert regions of Arizona, southern Nevada, and southeastern California. It is made up of a scattered growth of creosote bush and an abundance of cacti, especially the barrel cactus which varies in diameter from 1 to 1½ feet, and in height from 2 to 4 feet; and the round-stemmed *Opuntias*, especially the dense spiny *Opuntia bigelovii*. It is nonagricultural unless irrigated and of little or no value for grazing.

(76) **CREOSOTEBUSH-OCOTILLO** (*Fouquieria splendens* Engelm.).—The creosotebush-ocotillo type, which comprises a scattered growth of creosotebush and ocotillo, and many other plants of less conspicuous nature, occupies rocky and gravelly soils. It is widely distributed in the desert areas from southeastern California to western Texas. Both of the dominant species in this type are unpalatable to stock and the rocky nature of the habitat precludes the production of annuals. Lands producing this type are therefore nonagricultural and have a very low carrying capacity.

(77) **GIANT CACTUS** (*Carnegiea gigantea* (Engelm.) Britton & Rose), and **BUR-SAGE**.—Giant cactus and bur-sage are the dominant species in this type.

The former grows to a height of from 20 to 30 feet and is characterized by white straight spines. The type occurs in the desert regions of Arizona and California, where it is most frequently found on rocky ridges and foothills. It is of little or no grazing value and nonagricultural.

(78) **MESQUITE**.—Areas supporting little or no vegetative growth other than mesquite are classified in this type. This shrub, illustrated by Plate 13, B, varies in size from only a few feet to trees 30 feet high. It occupies a large acreage in southern New Mexico and Arizona and has considerable economic value for the stock feed supplied from the leaves and beans. The roots and the thicker stems also supply considerable fuel. Very common in deep fertile soil along drainage channels where subirrigation is usually received. The better types of this land are capable of producing forage crops such as sorghums, corn, and millet, during very favorable years. It has a carrying capacity of 5 to 15 head of cattle per section.

(79) **MESQUITE AND CHAMISO** (*Atriplex canescens* (Pursh) Nutt.).—All lands supporting a scattered growth of mesquite and chamiso, a shrub varying in height from 2 to 4 feet, are listed under this type. It is a very common type in southern New Mexico and Arizona, where it grows in clay loam soils. The mesquite trees are probably supported to a large extent by subsoil moisture. The agricultural possibilities of lands supporting this type are similar to those producing the preceding type. Chamiso is browsed extensively by cattle and sheep, making the type rather valuable for grazing. It has a carrying capacity of 20 to 30 head of cattle per section.

(80) **MESQUITE AND MESQUITE GRASS**.—The vegetation in this type consists of a scattered growth of mesquite over grassland which is usually composed of crowfoot grama, black grama, or grama-buffalo grass in the transition zone to this type, and in Texas buffalo curly mesquite (*Hilaria cenchroides* H. B. K.). It is usually characteristic of deep fertile loam soils. It is valuable grazing land, and where the grasses are mostly buffalo and curly mesquite, crops of cotton can be produced during the most favorable years. It has a carrying capacity of 20 to 30 head of cattle per section.

(81) *Parosela arborescens* (Torr.) Heller, and *Lepidospartum squamatum* A. Gray.—*Parosela* is a shrub from 3 to 6 feet high, with finely divided long leaves, the whole shrub presenting a misty or gray appearance and having deep indigo flowers. *Lepidospartum squamatum* is a rather low-growing shrub, harsh to the touch. This type is limited to the dry washes in southern Nevada and southeastern California and adjacent regions. Both species are worthless for grazing, and owing to the aridity of the region where they grow, crops can not be raised without irrigation.

(82) **PLUCHEA** (*Pluchea sericea* (Nutt.) Coville).—*Pluchea* is a tall straight-stemmed shrub, that seldom branches and grows in rather dense thickets. It was used to a considerable extent by the Indians to make arrows. This species occurs on strongly alkaline soil in limited quantities in western Arizona and southeastern California. It characterizes lands worthless for crop production and grazing.

(83) *Atriplex lentiformis* (Torr.) S. Wats.—*Atriplex lentiformis* is restricted to the low wet alkali lands of southeastern California, bordering salt lakes, particularly Salton Sink. It grows in large hummocklike clumps 6 to 15 feet across. There is usually little growth between these plants, which are widely spaced. Lands producing this type are incapable of crop production without leaching out the excess amount of alkali. It is grazed by stock mainly during periods of drought when little forage is available.

(84) **DESERT SAGE** (*Atriplex polycarpa* S. Wats.).—Desert sage, which is also known as saltbush and desert saltbush, illustrated by Plate 14, A, is limited largely to southeastern California, western Arizona, and southern Nevada.

It is a gray bush ranging from 2 to 5 feet in height, found mainly in chalky soils that contain more or less alkali. Dominant type on the bottom lands along Gila River, where it is found mainly in open stands. The type characterizes the most productive land of this region when it is placed under irrigation. It has a grazing value similar to that of *Atriplex lentiformis*.

(85) YUCCA DESERT GRASS.—Areas containing a scattered growth of yucca, usually *Yucca radiosa* (Englem.) Trel, over a grass cover composed usually of tobosa grass, are classified in this type. Yucca may also be distributed over areas characterized by *Bouteloua rothrockii* or *Bouteloua eriopoda*. It is common in New Mexico and eastern Arizona where it grows in rather loose open soils. Very little value for crop production without irrigation. The grasses in this type are palatable to livestock and the yucca is used to some extent as an emergency stock feed during droughts. The carrying capacity of this type is 15 to 25 head per section.

(86) BLACK BRUSH (*Flourensia cernua* DC.).—The black brush type contains few if any other species of vegetation. It grows in rather dense stands in well-drained porous soils in southern New Mexico and eastern Arizona. It has no value for crop production unless irrigated. It is also nonpalatable to all classes of livestock.

(87) BLACK BRUSH GRASS.—Plate 14, B, illustrates a typical area supporting this type, which is made up of a scattered stand of black brush and a little creosotebush with varying densities of grasses, consisting mainly of tobosa, black grama, and false needlegrass (*Scleropogon brevifolius* Phil.). This type is common in the semidesert regions of southern New Mexico and eastern Arizona. It characterizes lands that have little or no value for growing crops. With the exception of tobosa, which is only grazed when green, the other grasses are highly palatable to all classes of livestock. It has a carrying capacity of 10 to 20 head of cattle per section.

(88) NOLINA (*Nolina microcarpa* S. Wats.).—Nolina, also known as bear grass, produces a dense tufted bunched growth having very coarse foliage, varying in height from 2 to 4 feet. It is common in rather dry rocky localities in southern New Mexico and Arizona. It is not grazed owing to its tough character, and the localities where it grows are usually too rocky to have any value for producing crops.

(89) SHINNERY (*Quercus havardii* Rydb.).—This type, which is illustrated by Plate 15, A, is very common over the sandhills of New Mexico, Texas, and probably in western Oklahoma. It consists largely of an even distribution of the low-growing shinnery, seldom exceeding 2 feet in height. It often contains a scattered growth of mesquite. Browsed to a limited extent by livestock. It is very important in years of drought when other feed is very scarce. It is reported to be poisonous to cattle when the leaves are budding out. Shinnery lands are too sandy to be used for crop production.

(90) WILLOW (*Salix* sp.).—This type is restricted to narrow strips along creeks or other wet or moist places in the mountainous regions of the West, at elevations where the growing season is frequently too short to mature grain crops. Willows are also frequently found in small patches in wet meadows. Unless the willows are very dense a good undergrowth of sedges, rushes, and grasses is produced which are very palatable to sheep and cattle and thus give the land a high carrying capacity.

(91) MOUNTAIN BRUSH.—This type, shown by Plate 15, B, includes all brush land where chokecherry (*Prunus melanocarpa* (A. Nels.) Rydb.), snowberry (*Symphoricarpos* sp.), shadblow (*Amelanchier* sp.), oak brush (*Quercus utahensis* (A. DC.) Rydb.), scrub maple (*Acer* sp.), river hawthorn (*Crataegus rivularis* Nutt.), and sagebrush are the predominant shrubs, occurring either in equal quantities or with one predominating and with one or none of the

other species. Under the shrubs there is usually a good stand of grasses and weeds. This type is restricted to the mountain and foothill areas of the Rocky Mountains, about 6,000 feet elevation. With the exception of sagebrush, all the species are very good browse for cattle and sheep. Areas that can be cultivated are usually small and although lands of this character could be used for crop production their position and distribution are such that only small garden patches can be developed. The soil is usually dark and rich, but the growing season is sometimes too short to mature grains. The carrying capacity of this type is about 25 to 50 head of cattle per section.

(92) MOUNTAIN MAHOGANY (*Cercocarpus parvifolius* Nutt.).—Included in this type are all brush lands where mountain mahogany or closely related species predominate. It may be associated with oak brush, shadblow, river hawthorn, and mormon-tea. This type occupies drier soils than the preceding type. The stand of herbaceous vegetation under the shrubs is rather scattering. It occurs in rather low mountains and on foothills in Colorado, Utah and New Mexico. It is nonagricultural owing to the broken and rocky character of the surface but forms good browse for cattle and sheep, having a carrying capacity of 20 to 40 head of cattle per section.

(93) CHAPARRAL (*Ceanothus velutinus* Dougl.).—This is a very common brush type on burned-over timber lands, representing one of the stages of reproduction, where it often occurs in dense stands. It is also found on foothills associated with sagebrush, chokecherry, shadblow, aspen, or manzanita. It is a very common type above 6,000 feet in all the Western States except New Mexico and Arizona. It usually occupies lands too rough to be used for growing crops and it is also worthless for grazing.

(94) MANZANITA BRUSH TYPE.—This type includes all brush land where manzanitas (*Arctostaphylos manzanita* Parry, *A. pungens* H. B. K., *A. nevadensis* Parry) are the predominant shrubs. This type is very common in the mountain and foothill lands of Oregon and California, growing most commonly on southern slopes. In Oregon the manzanitas comprise 75 to 95 per cent of the shrubs in the type, while the remaining 5 to 25 per cent is made up of white oak (*Quercus garryana* Hook.), *Ceanothus cuneatus*, California laurel (*Arbutus menziesii* Pursh.) and black oak (*Quercus californica* (Torr.) Coop.). This type comes in on burned areas and is most frequently found in dense patches. Its agricultural possibilities are similar to the preceding type, and its carrying capacity is very low.

(95) SOUTHWEST MOUNTAIN BRUSH TYPE.—This is a mixed mountain and foothill brush type found in New Mexico and Arizona containing a predominance of one or all of the following shrubs: Catsclaw (*Acacia greggii* A. Gray), *Baccharis pteronioides* DC., small mesquite (*Mimosa biuncifera* Benth.), Apache plum (*Fallugia paradoxa* (D. Don) Endl.), mormon-tea (*Ephedra*), cliffrose, and shadblow (*Amelanchier* sp.). The last two species are usually found above 7,000 feet, while the others are found below this elevation. The lower species may have a scattering of scrub oak and mesquite. This is nonagricultural due to the broken and rocky character of the lands occupied by this type. It has a carrying capacity of 15 to 25 head of cattle per section.

(96) OAK BRUSH.—Classified under this type are all brush lands where oak brush or other closely related species predominate. This is a foothill type and is common in Utah, Colorado, New Mexico, and Arizona, where the oak brush consists mainly of *Quercus utahensis* (A. DC.) Rydb., and *Q. arizonica* Sarg. The latter species is restricted largely to Arizona and New Mexico. Associated with the oaks are the shrubs listed in Types (91) and (92) for Utah and Colorado and Type (95) for New Mexico and Arizona. In the coast region of northern California the oak brush type consists of a mixture of live oaks (*Quercus chrys-*

*olepis* Liebm., *Q. agrifolia* Nee, *Q. wislizenii* A.DC.) and scrub oak (*Quercus dumosa* Nutt.). This type grows mainly on foothill lands that are mainly non-tillable, consequently it has little importance for growing crops. It has a carrying capacity of 20 head of cattle per square mile.

(97) CHAMISE. (*Adenostoma fasciculatum* Hook. & Arn.)—Chamise, also known as chamiso, illustrated by Plate 16, A, is a brush-land type found in the foothills and mountain regions of California and southern Oregon up to about 2,500 feet elevation. This type grows in dense stands on dry, rather rocky, slopes in the coastal ranges of California below the conifer timber zone. This species is worthless for grazing and it grows on lands that can not be used for crop production owing mainly to their rocky and broken character.

(98) *Ceanothus cuneatus* Nutt.—This type, shown by Plate 16, B, occupies the drier mountain slopes below the conifer timber zone on the inland mountain ranges of California and on the west slopes of the Cascades in southern Oregon. In Oregon, this species is associated with white oak (*Q. garryana* Hook), manzanita, and Garrya (*Garrya elliptica* Dougl.). Under the shrubs is a scattering of annual weeds and grasses. It is browsed quite readily by cattle, goats, and sheep.

(99) CALIFORNIA MIXED BRUSH TYPE.—This is a California brush-land type in which there is a mixture of chaparral and other brush species, no one standing out predominantly. In the coastal mountain region of northern California, this type consists of a mixture of chamise, *Ceanothus cuneatus* Nutt., live oak, scrub oak, blue brush (*Ceanothus integerrimus* Hook & Arn.), bitter cherry (*Prunus emarginata* (Dougl.) Walp.), white thorn (*Ceanothus cordulatus* Kellogg), mountain mahogany, poison oak (*Rhus integrifolia* (Nutt.) Benth. & Hook), and pepper wood (*Umbellularia californica* (Hook & Arn.) Nutt.). This type occupies the better sites where the soil and moisture are more favorable for plant growth than the previous type. It has only a low value for grazing and the surface is too rolling and mountainous to be used for cultivation.

(100) ASPEN (*Populus tremuloides* Michx.).—This type includes all lands where aspen makes up 70 per cent or more of the stand of tree species. It often occurs in pure stands, but is frequently found in mixture with various conifers. In Colorado and Wyoming the conifers are usually Englemann spruce, alpine fir, Douglas fir, or yellow pine. In Arizona and New Mexico they are mainly Englemann spruce, corkbark pine, alpine fir, Douglas fir, and white fir. In Idaho and Utah the conifer is usually Douglas fir, lodgepole pine, white fir, and Englemann spruce. Aspen makes good fence posts, and in central Utah it has been used quite extensively for mine props. There is usually enough herbaceous vegetation growing under the aspens to make it very good grazing, especially for sheep. The frost-free season is usually too short to mature cultivated crops.

(101) WOODLAND TIMBER.—This type includes all areas in woodland timber. The main species classified as woodland are piñon, digger pine, and juniper. Very common in the western United States, along the foothills on rather dry southern exposures particularly in the Southwest. Very little herbaceous growth is produced in this type, and it has a low grazing value. It is of little value for agriculture, except in the more favorable localities where forage crops can be produced.

(102) CONIFER TIMBER.—Classified in this type are all lands where conifer timber, except the species classified as woodland, is growing in sufficient quantities to have a conifer timber aspect. The value of conifer timber land for grazing varies considerably, the amount of feed produced being governed mainly by the density of the stand. Conifer timber in the West is also mainly located at elevations too high for the maturity of cultivated crops.

KEY TO AGRICULTURAL POSSIBILITIES OF VEGETATION TYPES

The natural vegetation as a rule does not indicate favorable or unfavorable topography or the tillable or untillable character of the soil. It does, however, serve as an index to the potential possibilities of the habitat for growing other plants. A few of the types described in this paper may cover a large area, representing a considerable range in growing conditions. Any existing difference will be shown, however, in the density and thrift of the stand of vegetation even where there are not any special plants present to indicate changes. On a large area of the range lands of the West, especially the unreserved public domain, overgrazing has changed the natural stand of vegetation to such an extent that the plants now growing on the land can not be used as an indicator of the agricultural possibilities. Overgrazed areas can be readily identified by the replacement of the more tender and succulent plants by tougher and non-palatable species and by the roots of the vegetation being exposed by tramping.

In the following key the types are listed roughly in order of the value of the lands they occupy for the production of cultivated crops. Since some of the types cover a wide range of growing conditions, they may be found on locations better or poorer than are indicated by this key.

The types are listed in two general classes: (1) Those indicating favorable possibilities for growing crops by dry farming, and (2) those indicating lands where cultivated crops can not be successfully produced by dry farming and are therefore mainly valuable for grazing. The types indicating favorable conditions for dry farming are also divided into two classes: (a) Those that indicate favorable possibilities for producing grain cereals, and (b) those indicating lands that are suitable for the production of forage crops. Each of these groups is further divided into three general classes—best, medium, and poorest types.

1. DRY FARM LAND.—Lands which under proper cultivation are capable of producing crops without irrigation more valuable than the natural vegetation.

(a) *Grain land*.—Types indicating lands suitable for the production of small grains.

Best types:

- |  |                                   |
|--|-----------------------------------|
| (11) Grama grass and valley sage.          | (7) Grama grass and sagebrush.    |
| (12) Grama grass and western needle-grass. | (9) Grama grass and wild alfalfa. |
| (24) Western needlegrass.                  | (91) Mountain brush land.         |
| (5) Grama grass and mountain sage.         | (40) Curly bunchgrass.            |

Medium types:

- |                                      |  |
|--------------------------------------|--|
| (42) Arizona fescue.                 | (4) Grama grass, niggerwool, and Junegrass.  |
| (39) Wheat bunchgrass.               | (45) Weed grass.                             |
| (17) Grama-buffalo and wild alfalfa. | (15) Grama-buffalo and wire needle-grass.    |
| (47) Dry meadow.                     | (14) Grama-buffalo and western needle-grass. |
| (53) Antelope-brush.                 | (2) Grama grass and wheatgrass.              |
| (22) Wire needlegrass.               | (96) Oak brush.                              |
| (13) Grama-buffalo grass.            |  |
| (16) Grama-buffalo and wheatgrass.   |  |

Poorest types:

- |                                 |                          |
|---------------------------------|--------------------------|
| (49) Sagebrush.                 | (56) Little rabbitbrush. |
| (21) Wheatgrass.                | (1) Grama grass.         |
| (3) Grama grass and niggerwool. | (44) Giant wild-rye.     |

(b) *Forage land*.—Lands which, under cultivation, are suitable in most localities for the production of forage crops, such as corn, sorghums, millet, cow peas, or small grains cut for forage.

Best types:

- |   |   |
|---|---|
| (29) Bunchgrass.                              | (90) Willows.                           |
| (100) Aspen.                                  | (28) Hairy grama, grama, and sand sage. |
| (48) Mountain weed.                           | (19) Grama-buffalo and mesquite.        |
| (41) Mixed mountain grass land.               | (80) Mesquite and mesquite grass.       |
| (20) Grama-buffalo and matchweed.             | (10) Grama grass and matchweed.         |
| (18) Grama-buffalo grass and soap-weed yucca. |   |

Medium types:

- |                              |                                     |
|------------------------------|-------------------------------------|
| (30) Sand hills mixed.       | (95) Southwest mountain brush land. |
| (27) Black grama.            | (102) Conifer timber.               |
| (8) Grama grass and chamiso. | (101) Woodland timber.              |
| (35) Crowfoot grama.         |                                     |

Poorest types:

- |   |   |
|---|---|
| (89) Shinnery.                          | (6) Grama grass, niggerwool, and sagebrush. |
| (32) Black grama and tobosa grass.      | (23) Niggerwool.                            |
| (33) Tobosa grass.                      | (85) Yucca desert grass land.               |
| (31) Black grama and wire needle-grass. | (98) <i>Ceanothus cuneatus</i> .            |

2. *GRAZING LANDS*.—Lands either unsuitable for the production of cultivated crops by arid farming or on which the production of native vegetation is more valuable than the crops that can be produced by arid farming.

Best types:

- |                     |                                     |
|---------------------|-------------------------------------|
| (46) Wet meadow.    | (64) Greasewood and saltgrass.      |
| (34) Galleta.       | (57) Rabbitbrush and tussock grass. |
| (38) Sacaton.       | (92) Mountain mahogany.             |
| (37) Tussock grass. | (58) Winter-fat.                    |
| (65) Saltgrass.     |                                     |

Medium types:

- |                                 |                                    |
|---------------------------------|------------------------------------|
| (78) Mesquite.                  | (63) Greasewood-saltsage.          |
| (79) Mesquite and chamiso.      | (99) California mixed brush type.  |
| (54) Shadscale.                 | (73) Desert brush type.            |
| (55) Salt sage.                 | (83) <i>Atriplex lentiformis</i> . |
| (74) Creosotebush-desert grass. | (61) Greasewood.                   |
| (87) Black brush grass land.    | (52) Hop-sage.                     |
| (62) Greasewood-shadscale.      | (36) <i>Hilaria rigida</i> .       |

Poorest types:

- |  |   |
|--|---|
| (88) Nolina.                           | (66) Pickleweed.  |
| (50) Small sagebrush.                  | (67) Seepweed.  |
| (51) Scab sagebrush.                   | (68) Samphire.  |
| (69) Creosotebush.                     | (82) Pluchea.   |
| (97) Chamise.                          | (81) <i>Parosela arborescens</i> and <i>Lepidospartum squamatum</i> . |
| (76) Creosotebush-ocotillo.            | (77) Giant cactus and bur-sage.                                       |
| (75) Creosotebush and cacti.           | (60) Coleogyne.   |
| (72) Shrub buckwheat.                  | (84) Desert sage.   |
| (71) Encelia and California sagebrush. | (86) Black brush.   |
| (59) White sage.                       |   |
| (70) Creosotebush and bur-sage.        |   |

Types which do not indicate natural conditions, but come in as the result of cultivation, burning, or over-grazing:

- |                                   |                              |
|-----------------------------------|------------------------------|
| (26) Matchweed and mountain sage. | (94) Manzanita brush type.   |
| (45) Weed grass.                  | (25) Matchweed.              |
| (93) Chaparral.                   | (43) Short-lived grass type. |

## KEY TO THE CARRYING CAPACITY OF THE VEGETATION TYPES

Practically all types of vegetation are used to some extent for grazing. Even in regions where sufficient moisture falls to produce crops and where the soils are suitable for this purpose, there is always a large acreage of physically non-tillable land that can be used only for grazing stock. In classifying the types of vegetation according to carrying capacity they have been listed in the approximate order of their carrying capacity in three groups: (1) Best, (2) medium, and (3) poor to worthless. The carrying capacity figures are intended to cover the bulk of the lands occupied by each type but there may be areas having greater or lower carrying capacities for some of the types than are listed here. The carrying capacities also vary from year to year depending upon growing conditions. The figures given in this paper represent the carrying capacity in an average year. The figures also represent the carrying capacity for the period of each year that the lands occupied by the types can be grazed without injury to the vegetation. Unless otherwise stated the grazing capacity figures apply to the number of cattle that can be grazed on a square mile or section of land.

### BEST TYPES

Vegetation type:	Carrying capacity
(46) Wet meadow.....	100-300
(90) Willows.....	75-150
(65) Saltgrass.....	50-150
(38) Sacaton.....	50-100
(37) Tussock grass.....	50-100
(48) Mountain weed (sheep).....	300-600
(47) Dry meadow.....	50-100
(34) Galletagrass.....	25-100
(9) Grama grass and wild alfalfa.....	50- 90
(12) Grama grass and western needlegrass.....	40- 80
(19) Grama-buffalo and mesquite.....	40- 60
(14) Grama-buffalo and western needlegrass.....	35- 75
(16) Grama-buffalo and wheatgrass.....	30- 75
(41) Mixed mountain grass land.....	25- 75
(100) Aspen.....	30- 65
(17) Grama-buffalo and wild alfalfa.....	30- 65
(2) Grama grass and wheatgrass.....	30- 60
(11) Grama grass and valley sage.....	30- 60
(4) Grama grass, niggerwool, and Junegrass.....	30- 60
(40) Curly bunchgrass.....	30- 60
(39) Wheat bunchgrass.....	25- 60
(13) Grama-buffalo grass.....	25- 50
(5) Grama grass and mountain sage.....	25- 50
(18) Grama-buffalo grass and soapweed yucca.....	25- 50
(91) Mountain brush land.....	25- 50



## MEDIUM TYPES

	Carrying capacity
(30) Sand hills mixed.....	20-50
(1) Grama grass.....	20-40
(24) Western needlegrass.....	20-40
(3) Grama grass and niggerwool.....	20-40
(57) Rabbitbrush and tussock grass.....	20-40
(15) Grama-buffalo and wire needlegrass.....	20-40
(29) Bunchgrass.....	20-40
(21) Wheatgrass.....	10-50
(92) Mountain mahogany.....	15-40
(35) Crowfoot grama.....	20-35
(42) Arizona fescue.....	20-30
(53) Antelopebrush.....	20-30
(7) Grama grass and sagebrush.....	20-30
(80) Mesquite and mesquite grass.....	20-30
(96) Oak brush.....	15-30
(45) Weed grass.....	15-30
(27) Black grama.....	15-30
(31) Black grama and wire needlegrass.....	15-30
(8) Grama grass and chamiso.....	15-30
(64) Greasewood-saltgrass.....	10-40
(102) Conifer timber.....	10-30
(10) Grama grass and matchweed.....	15-25
(22) Wire needlegrass.....	15-25
(28) Hairy grama, grama, and sand sage.....	15-25
(32) Black grama and tobosagrass.....	15-25
(85) Yucca desert grass land.....	15-25
(95) Southwest mountain brush land.....	15-25
(49) Sagebrush.....	15-25
(44) Giant wild-rye.....	15-25
(79) Mesquite and chamiso.....	10-30
(101) Woodland timber.....	10-25
(87) Black brush grass land.....	10-20
(74) Creosotebush-desert grass.....	10-20
(99) California mixed brush type.....	10-20
(89) Shinnery.....	10-20
(58) Winter-fat (sheep).....	50-100
(54) Shadscale (sheep).....	25-100
(55) Salt sage (sheep).....	25-75
(62) Greasewood-shadscale (sheep).....	25-75
(63) Greasewood-salt sage (sheep).....	25-75
(33) Tobosa grass.....	10-20
(23) Niggerwool.....	5-15
(43) Short-lived grass type.....	5-15
(94) Manzanita brush type.....	5-15
(98) <i>Ceanothus cuneatus</i> .....	5-15
(10) Grama grass and matchweed.....	5-15
(26) Matchweed and mountain sage.....	5-15
(88) Nolina.....	5-15
(93) Chaparral.....	5-15
(56) Little rabbitbrush.....	5-15
(73) Desert brush type.....	5-12
(78) Mesquite.....	5-10
(50) Small sagebrush.....	5-10
(61) Greasewood (sheep).....	25-50

## POOR TO WORTHLESS TYPES

	Carrying capacity
(36) <i>Hilaria rigida</i> .....	5-10
(25) Matchweed.....	5-10
(84) Desert-sage.....	10-
(51) Scab sagebrush.....	5-10
(69) Creosotebush.....	0-10
(86) Black brush.....	0-10
(75) Creosotebush and cacti.....	0-10
(70) Creosotebush and bur-sage.....	0-10

## LITTLE OR NO GRAZING VALUE

(59) White sage.	(76) Creosotebush-ocotillo.
(60) Coleogyne.	(77) Giant cactus and bur-sage.
(66) Pickleweed.	(81) <i>Parosela arborescens</i> and <i>Lepidospartum squamatum</i> .
(67) Seepweed.	(82) Pluchea.
(68) Samphire.	(83) <i>Atriplex lentiformis</i> .
(71) Encelia and California sagebrush.	
(72) Shrub buckwheat.	

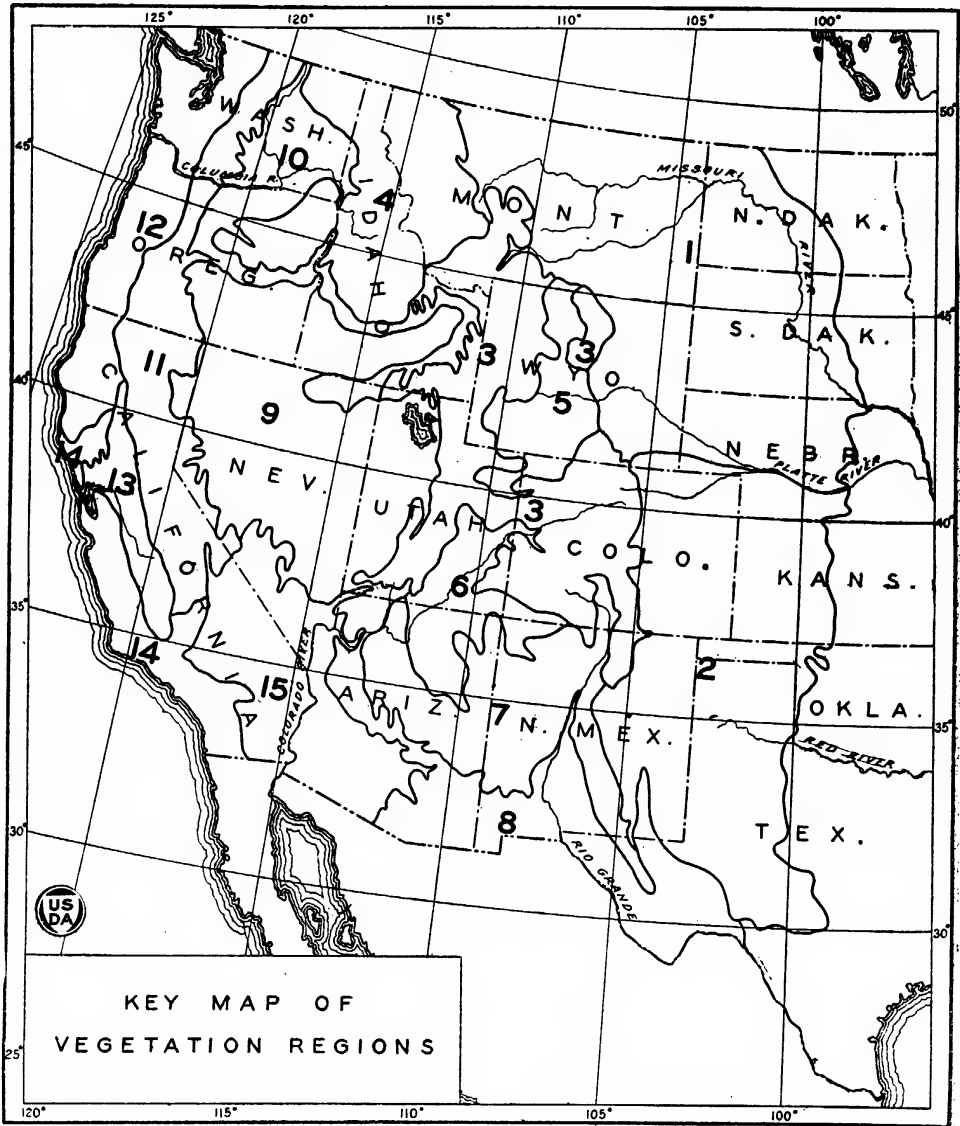
## TYPES OF VEGETATION GROUPED BY REGION

The following is a list of the types of vegetation, giving their distribution in the regions outlined on the key map of the vegetation regions. The regions listed are believed to include practically the entire range for a large percentage of the types. A few, however, may extend to a limited extent into adjoining regions.

Type	Regions
(1) Grama grass ( <i>Bouteloua gracilis</i> ).....	1, 2, 3, 7, 8
(2) Grama grass and wheatgrass ( <i>Agropyron smithii</i> ).....	1, 2, 7
(3) Grama grass and niggerwool ( <i>Carex filifolia</i> ).....	1, 2, 5, 7
(4) Grama grass, niggerwool, and Junegrass ( <i>Koeleria cristata</i> ).....	1, 2, 7
(5) Grama grass and mountain sage ( <i>Artemisia frigida</i> ).....	1, 2
(6) Grama grass, niggerwool, and sagebrush ( <i>Artemisia tridentata</i> ).....	1, 5
(7) Grama grass and sagebrush.....	3, 5, 7
(8) Grama grass and chamiso ( <i>Atriplex canescens</i> ).....	1, 2, 3, 5, 7
(9) Grama grass and wild alfalfa ( <i>Psoralea tenuiflora</i> ).....	1, 2
(10) Grama grass and matchweed ( <i>Gutierrezia sarothrae</i> ).....	1, 2, 3, 5, 7
(11) Grama grass and valley sage ( <i>Artemisia nana</i> ).....	1
(12) Grama grass and western needlegrass ( <i>Stipa comata</i> ).....	1
(13) Grama-buffalo grass ( <i>Bulbilis dactyloides</i> ).....	1, 2
(14) Grama-buffalo grass and western needlegrass.....	1, 2
(15) Grama-buffalo grass and wire needlegrass ( <i>Aristida longiseta</i> ).....	2
(16) Grama-buffalo and wheatgrass.....	1, 2
(17) Grama-buffalo and wild alfalfa.....	1, 2
(18) Grama-buffalo grass and soapweed yucca ( <i>Yucca glauca</i> ).....	2
(19) Grama-buffalo and mesquite ( <i>Prosopis glandulosa</i> ).....	2
(20) Grama-buffalo grass and matchweed.....	1, 2
(21) Wheatgrass.....	1
(22) Wire needlegrass.....	2, 7, 8
(23) Niggerwool.....	1, 5
(24) Western needlegrass.....	1, 2
(25) Matchweed.....	1, 2, 3, 7, 8
(26) Matchweed and mountain sage ( <i>Artemisia frigida</i> ).....	1
(27) Black grama ( <i>Bouteloua eriopoda</i> ).....	8
(28) Hairy grama ( <i>Bouteloua hirsuta</i> ), grama, and sand sage ( <i>Artemisia filifolia</i> ).....	2
(29) Bunchgrass ( <i>Andropogon scoparius</i> ).....	1, 2
(30) Sand hills mixed.....	1, 2

Type	Regions
(31) Black grama and wire needlegrass	8
(32) Black grama and tobosa grass ( <i>Hilaria mutica</i> )	8
(33) Tobosa grass	2, 7, 8
(34) Galleta grass ( <i>Hilaria jamesii</i> )	5, 6, 7, 8, 9
(35) Crowfoot grama ( <i>Bouteloua rothrockii</i> )	7, 8
(36) <i>Hilaria rigida</i>	15
(37) Tussock grass ( <i>Sporobolus airoides</i> )	2, 5, 6, 7, 8, 9, 15
(38) Sacaton ( <i>Sporobolus wrightii</i> )	2, 6, 7, 8
(39) Wheat bunchgrass ( <i>Agropyron spicatum</i> )	10
(40) Curly bunchgrass ( <i>Festuca ovina</i> )	3, 10
(41) Mixed mountain grass	3, 10
(42) Arizona fescue ( <i>Festuca arizonica</i> )	7
(43) Short-lived grass type	8
(44) Giant wild rye ( <i>Elymus condensatus</i> )	3, 9
(45) Weed grass	12, 13, 14
(46) Wet meadow	3, 4, 7, 10, 11, 12, 14
(47) Dry meadow	3, 4, 10, 11, 12, 14
(48) Mountain weed	3, 4, 10, 11, 12
(49) Sagebrush ( <i>Artemisia tridentata</i> )	3, 5, 6, 7, 9, 10, 11
(50) Small sagebrush ( <i>Artemisia nova</i> )	3, 5, 6, 9
(51) Scab sagebrush ( <i>Artemisia rigida</i> )	10
(52) Hop-sage ( <i>Grayia spinosa</i> )	5, 6, 9
(53) Antelope-brush ( <i>Purshia tridentata</i> )	3, 10
(54) Shadscale ( <i>Atriplex confertifolia</i> )	5, 6, 9
(55) Salt sage ( <i>Atriplex corrugata</i> and <i>A. nuttallii</i> )	5, 9
(56) Little rabbitbrush ( <i>Chrysothamnus stenophyllus</i> )	9
(57) Rabbitbrush ( <i>Chrysothamnus graveolens</i> ) and tussock grass	2
(58) Winter-fat ( <i>Eurotia lanata</i> )	5, 6, 9
(59) White sage ( <i>Kochia americana</i> )	5, 9
(60) Coleogyne ( <i>Coleogyne ramosissima</i> )	9, 15
(61) Greasewood ( <i>Sarcobatus vermiculatus</i> )	5, 6, 8, 9
(62) Greasewood, Shadscale	5, 6, 9
(63) Greasewood, Salt sage	5, 6, 9
(64) Greasewood, Saltgrass ( <i>Distichlis spicata</i> )	5, 6, 7, 9
(65) Saltgrass	1, 2, 5, 6, 7, 8, 9, 13, 15
(66) Pickleweed ( <i>Allenrolfea occidentalis</i> )	8, 9, 15
(67) Seepweed ( <i>Dondia moquini</i> )	5, 6, 9
(68) Samphire ( <i>Salicornia utahensis</i> and <i>S. rubra</i> )	5, 6, 9
(69) Creosotebush ( <i>Covillea glutinosa</i> )	8, 15
(70) Creosotebush and bur-sage ( <i>Franseria deltoidea</i> , <i>Franseria dumosa</i> )	15
(71) Encelia ( <i>Encelia farinosa</i> ) and California sagebrush	13, 15
(72) Shrub buckwheat ( <i>Eriogonum fasciculatum</i> )	9, 15
(73) Desert-brush type: Creosotebush, ironwood ( <i>Olneya tesota</i> ), mesquite, palo verde ( <i>Parkinsonia microphylla</i> ), and catsclaw ( <i>Acacia greggii</i> )	15
(74) Creosotebush-desert grass	2, 8
(75) Creosotebush and cacti ( <i>Opuntia</i> species)	15
(76) Creosotebush-ocotillo ( <i>Fouquieria splendens</i> )	8, 15
(77) Giant cactus ( <i>Carnegiea gigantea</i> ) and bur-sage	15
(78) Mesquite	2, 8, 15
(79) Mesquite and chamiso ( <i>Atriplex canescens</i> )	8
(80) Mesquite and mesquite grass	8
(81) <i>Parosela arborescens</i> and <i>Lepidospartum squamatum</i>	15
(82) Pluchea ( <i>Pluchea sericea</i> )	15
(83) <i>Atriplex lentiformis</i>	15

Type	Regions
(84) Desert-sage ( <i>Atriplex polycarpa</i> )	15
(85) Yucca desert grass	8
(86) Black brush ( <i>Flourensia cernua</i> )	8
(87) Black brush grass	8
(88) Nolina ( <i>Nolina microcarpa</i> )	8
(89) Shinnery ( <i>Quercus havardii</i> )	2
(90) Willow ( <i>Salix</i> species)	3, 4, 7, 10, 11, 12, 13
(91) Mountain brush	3, 10
(92) Mountain mahogany ( <i>Cercocarpus parvifolius</i> )	3, 7
(93) Chaparral ( <i>Ceanothus velutinus</i> )	3, 4, 11
(94) Manzanita—brush type	12, 14
(95) Southwest mountain brush	7, 8
(96) Oak brush	3, 7, 8
(97) Chamise ( <i>Adenostoma fasciculatum</i> )	14
(98) <i>Ceanothus cuneatus</i>	11, 12, 14
(99) California mixed brush type	11, 12, 14
(100) Aspen ( <i>Populus tremuloides</i> )	1, 3, 4, 7
(101) Woodland timber	1, 2, 3, 6, 7, 8, 9, 10, 11, 14
(102) Conifer timber	1, 2, 3, 4, 5, 7, 8, 10, 11, 12, 14



## DISTRIBUTION OF THE TYPES IN THE 15 VEGETATION REGIONS

## REGION 1

- |   |   |
|---|---|
| (1) Grama grass.                                  | (16) Grama-buffalo and wheatgrass.†     |
| (2) Grama grass and wheatgrass.                   | (17) Grama-buffalo and wild alfalfa.    |
| (3) Grama grass and niggerwool.                   | (20) Grama-buffalo grass and matchweed. |
| (4) Grama grass, niggerwool, and June-grass.      | (21) Wheatgrass.                        |
| (5) Grama grass and mountain sage.                | (23) Niggerwool.                        |
| (6) Grama grass, niggerwool, and sagebrush.       | (24) Western needlegrass.               |
| (8) Grama grass and chamiso.                      | (25) Matchweed.                         |
| (9) Grama grass and wild alfalfa.                 | (26) Matchweed and mountain sage.       |
| (10) Grama grass and matchweed.                   | (29) Bunchgrass.                        |
| (11) Grama grass and valley sage.                 | (30) Sand hills mixed.                  |
| (12) Grama grass and western needlegrass.         | (65) Saltgrass.                         |
| (13) Grama-buffalo grass.                         | (100) Aspen.                            |
| (14) Grama-buffalo grass and western needlegrass. | (101) Woodland timber.                  |
|   | (102) Conifer timber.                   |

## REGION 2

- |   |   |
|---|---|
| (1) Grama grass.                                  | (20) Grama-buffalo grass and matchweed. |
| (2) Grama grass and wheatgrass.                   | (22) Wire needlegrass.                  |
| (3) Grama grass and niggerwool.                   | (24) Western needlegrass.               |
| (4) Grama grass, niggerwool, and June-grass.      | (25) Matchweed.                         |
| (5) Grama grass and mountain sage.                | (28) Hairy grama, grama, and sand sage. |
| (8) Grama grass and chamiso.                      | (29) Bunchgrass.                        |
| (9) Grama grass and wild alfalfa.                 | (30) Sand hills mixed.                  |
| (10) Grama grass and matchweed.                   | (33) Tobosa grass.                      |
| (13) Grama-buffalo grass.                         | (37) Tussock grass.                     |
| (14) Grama-buffalo grass and western needlegrass. | (38) Sacaton.                           |
| (15) Grama-buffalo grass and wire needlegrass.    | (57) Rabbitbrush and tussock grass.     |
| (16) Grama-buffalo and wheatgrass.                | (65) Saltgrass.                         |
| (17) Grama-buffalo and wild alfalfa.              | (74) Creosotebush-desert grass.         |
| (18) Grama-buffalo grass and soapweed yucca.      | (78) Mesquite.                          |
| (19) Grama-buffalo and mesquite.                  | (89) Shinnery.                          |
|   | (101) Woodland timber.                  |
|   | (102) Conifer timber.                   |

## REGION 3

- |                                 |                                      |
|---------------------------------|--------------------------------------|
| (1) Grama grass.                | (49) Sagebrush.                      |
| (7) Grama grass and sagebrush.  | (50) Small sagebrush.                |
| (8) Grama grass and chamiso.    | (53) Antelope-brush.                 |
| (10) Grama grass and matchweed. | (90) Willow ( <i>Salix</i> species). |
| (25) Matchweed.                 | (91) Mountain brush.                 |
| (40) Curly bunchgrass.          | (92) Mountain mahogany.              |
| (41) Mixed mountain grass.      | (93) Chaparral.                      |
| (44) Giant wild-rye.            | (96) Oak brush.                      |
| (46) Wet meadow.                | (100) Aspen.                         |
| (47) Dry meadow.                | (101) Woodland timber.               |
| (48) Mountain weed.             | (102) Conifer timber.                |

## REGION 4

- |                                      |                       |
|--------------------------------------|-----------------------|
| (46) Wet meadow.                     | (93) Chaparral.       |
| (47) Dry meadow.                     | (100) Aspen.          |
| (48) Mountain weed.                  | (102) Conifer timber. |
| (90) Willow ( <i>Salix</i> species). |                       |

## REGION 5

- |  |                            |
|--|----------------------------|
| (3) Grama grass and niggerwool.            | (54) Shadscale.            |
| (6) Gramagrass, niggerwool, and sagebrush. | (55) Salt sage.            |
| (7) Grama grass and sagebrush.             | (58) Winter-fat.           |
| (8) Grama grass and chamiso.               | (59) White sage.           |
| (10) Grama grass and matchweed.            | (61) Greasewood.           |
| (23) Niggerwool.                           | (62) Greasewood-shadscale. |
| (34) Galleta grass.                        | (63) Greasewood-salt sage. |
| (37) Tussock grass.                        | (64) Greasewood-saltgrass. |
| (49) Sagebrush.                            | (65) Saltgrass.            |
| (50) Small sagebrush.                      | (67) Seepweed.             |
| (52) Hop-sage.                             | (68) Samphire.             |
|  | (102) Conifer timber.      |

## REGION 6

- |                     |                            |
|---------------------|----------------------------|
| (34) Galleta.       | (61) Greasewood.           |
| (37) Tussock grass. | (62) Greasewood-shadscale. |
| (38) Sacaton.       | (63) Greasewood-salt sage. |
| (49) Sagebrush.     | (64) Greasewood-saltgrass. |
| (50) Small sage.    | (65) Saltgrass.            |
| (52) Hop sage.      | (67) Seepweed.             |
| (54) Shadscale.     | (68) Samphire.             |
| (58) Winter fat.    | (101) Woodland timber.     |

## REGION 7

- |   |                                      |
|---|--------------------------------------|
| (1) Grama grass.                            | (38) Sacaton.                        |
| (2) Grama grass and wheatgrass.             | (42) Arizona fescue.                 |
| (3) Grama grass and niggerwool.             | (46) Wet meadow.                     |
| (4) Gramagrass, niggerwool, and June-grass. | (49) Sagebrush.                      |
| (7) Grama grass and sagebrush.              | (64) Greasewood-saltgrass.           |
| (8) Grama grass and chamiso.                | (65) Saltgrass.                      |
| (10) Grama grass and matchweed.             | (90) Willow ( <i>Salix</i> species). |
| (22) Wire needlegrass.                      | (92) Mountain mahogany.              |
| (25) Matchweed.                             | (95) Southwest mountain brush        |
| (33) Tobosa grass.                          | (96) Oak brush.                      |
| (34) Galleta grass.                         | (100) Aspen.                         |
| (35) Crowfoot grama.                        | (101) Woodland timber.               |
| (37) Tussock grass.                         | (102) Conifer timber.                |

## REGION 8

- |   |                              |
|---|------------------------------|
| (1) Grama grass.                        | (34) Galleta grass.          |
| (22) Wire needlegrass.                  | (35) Crowfoot grama.         |
| (25) Matchweed.                         | (37) Tussock grass.          |
| (27) Black grama.                       | (38) Sacaton.                |
| (31) Black grama and wire needle-grass. | (43) Short-lived grass type. |
| (32) Black grama and tobosa grass.      | (61) Greasewood.             |
| (33) Tobosa grass.                      | (65) Saltgrass.              |

## REGION 8—Continued

- |                                   |                                |
|-----------------------------------|--------------------------------|
| (66) Pickleweed.                  | (85) Yucca desert grass.       |
| (69) Creosotebush.                | (86) Black brush.              |
| (74) Creosotebush-desert grass.   | (87) Black brush grass.        |
| (76) Creosotebush-ocotillo.       | (95) Southwest mountain brush. |
| (78) Mesquite.                    | (96) Oak brush.                |
| (79) Mesquite and chamiso.        | (101) Woodland timber.         |
| (80) Mesquite and mesquite grass. | (102) Conifer timber.          |

## REGION 9

- |                          |                            |
|--------------------------|----------------------------|
| (34) Galleta grass.      | (60) Coleogyne.            |
| (37) Tussock grass.      | (61) Greasewood.           |
| (44) Giant wild-rye.     | (62) Greasewood-shadscale. |
| (49) Sagebrush.          | (63) Greasewood-salt sage. |
| (50) Small sagebrush.    | (64) Greasewood-saltgrass. |
| (52) Hop-sage.           | (65) Saltgrass.            |
| (54) Shadscale.          | (66) Pickleweed.           |
| (55) Salt sage.          | (67) Seepweed.             |
| (56) Little rabbitbrush. | (68) Samphire.             |
| (58) Winter-fat.         | (72) Shrub buckwheat.      |
| (59) White sage.         | (101) Woodland timber.     |

## REGION 10

- |                            |                        |
|----------------------------|------------------------|
| (39) Wheat bunchgrass.     | (51) Scab sagebrush.   |
| (40) Curly bunchgrass.     | (53) Antelope-brush.   |
| (41) Mixed mountain grass. | (90) Willows.          |
| (46) Wet meadow.           | (91) Mountain brush.   |
| (47) Dry meadow.           | (101) Woodland timber. |
| (48) Mountain weed.        | (102) Conifer timber.  |
| (49) Sagebrush.            |                        |

## REGION 11

- |                     |                                   |
|---------------------|-----------------------------------|
| (46) Wet meadow.    | (93) Chaparral.                   |
| (47) Dry meadow.    | (98) <i>Ceanothus cuneatus</i> .  |
| (48) Mountain weed. | (99) California mixed brush type. |
| (49) Sagebrush.     | (101) Woodland timber.            |
| (90) Willows.       | (102) Conifer timber.             |

## REGION 12

- |                     |                                   |
|---------------------|-----------------------------------|
| (45) Weed grass.    | (94) Manzanita.                   |
| (46) Wet meadow.    | (98) <i>Ceanothus cuneatus</i> .  |
| (47) Dry meadow.    | (99) California mixed brush type. |
| (48) Mountain weed. | (102) Conifer timber.             |
| (90) Willows.       |                                   |

## REGION 13

- |                  |  |
|------------------|--|
| (45) Weed grass. | (71) Encelia and California sagebrush. |
| (65) Saltgrass.  | (90) Willows.                          |

## REGION 14

- |                            |                                   |
|----------------------------|-----------------------------------|
| (45) Weed grass.           | (98) <i>Ceanothus cuneatus</i> .  |
| (46) Wet meadow.           | (99) California mixed brush type. |
| (47) Dry meadow.           | (101) Woodland timber.            |
| (94) Manzanita—brush type. | (102) Conifer timber.             |
| (97) Chamise.              |                                   |

REGION 15

- |  |   |
|--|---|
| (36) <i>Hilaria rigida</i> .             | (73) Desert brush type.                             |
| (37) Tussock grass.                      | (75) Creosotebush and cacti.                        |
| (60) Coleogyne.                          | (76) Creosotebush-ocotillo.                         |
| (65) Saltgrass.                          | (77) Giant cactus and bur-sage.                     |
| (66) Pickleweed.                         | (78) Mesquite.                                      |
| (69) Creosotebush.                       | (81) <i>Parosela arborescens</i> and <i>Lepido-</i> |
| (70) Creosotebush and bur-sage.          | <i>spartum squamatum</i> .                          |
| (71) <i>Encelia</i> and California sage- | (82) <i>Pluchea</i> .                               |
| brush.                                   | (83) <i>Atriplex lentiformis</i> .                  |
| (72) Shrub buckwheat.                    | (84) Desert sage.                                   |



**PLATE 1<sup>a</sup>**

- A.-(1) Grama grass. East side Estancia Valley, N. Mex.**  
**B.-(5) Grama grass and mountain sage. Havre, Mont.**

<sup>a</sup> All photographs supplied by H. L. Shantz.





**PLATE 2**

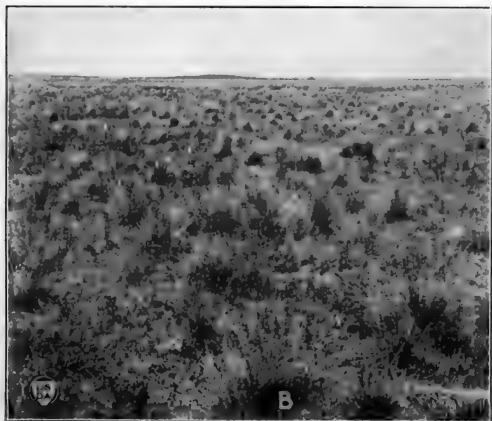
**A.-(6) Grama grass, niggerwool, and sagebrush. Storey, Wyo.**

**B.-(11) Grama grass and valley sage. Havre, Mont.**

**PLATE 3**

**A.-(13) Grama-buffalo grass. East of Limon, Colo.**

**B.-(15) Grama-buffalo and wire needlegrass. Yuma, Colo.**



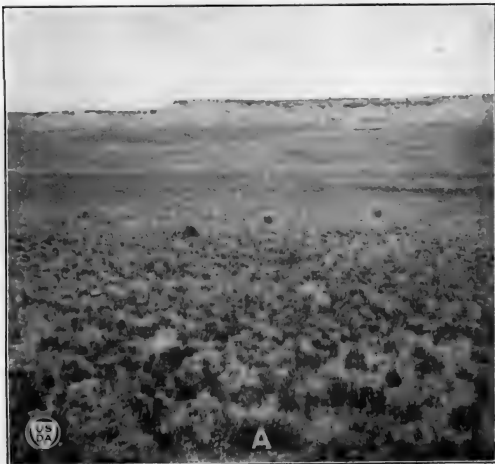


PLATE 4

- A.-(20) Grama-buffalo grass and Gutierrezia. Southeast of Hartley, Tex.  
B.-(30) Sand hills mixed. Wray, Colo.

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**PLATE 5**

**A.-(37) Tussock grass. 15.2 miles south of Roswell, N. Mex.**

**B.-(39) Wheat bunchgrass. Heppner, Oreg.**





**PLATE 6**

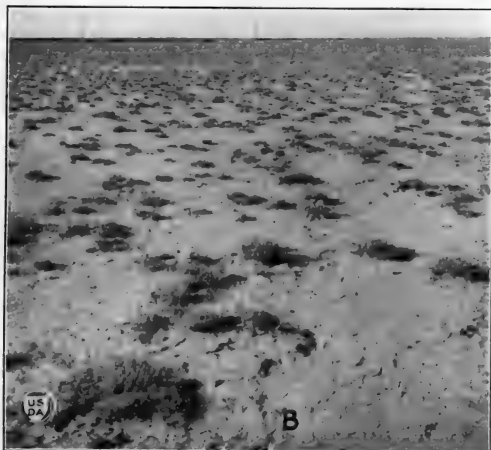
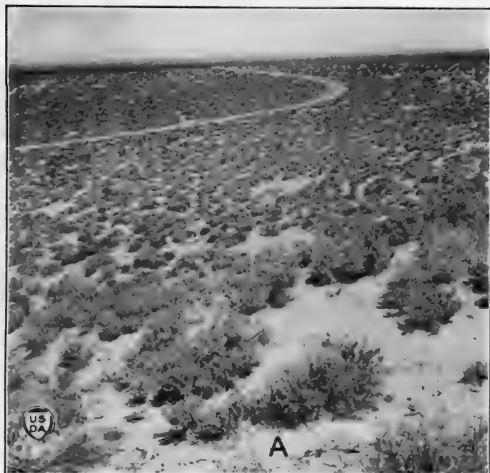
**A.-(44) Giant wild-rye. Harper, Oreg.**

**B.-(49) Sagebrush. Elko, Nev.**

**PLATE 7**

- A.-(50) Small sagebrush      Burns, Oreg.**  
**B.-(53) Antelope-brush.      Millikin Valley, Oreg.**





**PLATE 8**

A.-(54) Shadscale. Hollow beyond Grantsville; Utah.

B.-(55) Salt sage. Lund, Utah.



**PLATE 9**

**A.-(56) Little rabbitbrush. Modena, Utah.**

**B.-(58) Winter-fat. Near Modena, Utah.**





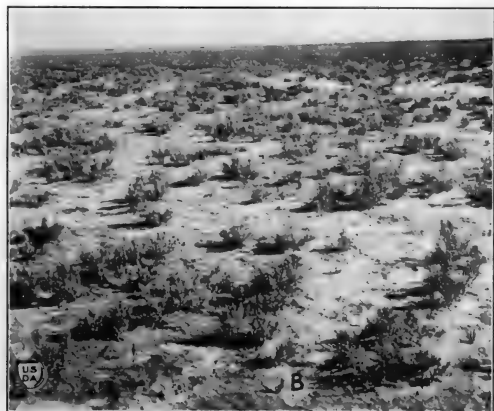
**PLATE 10**

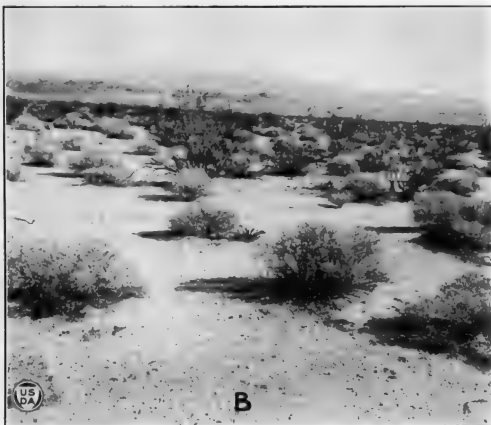
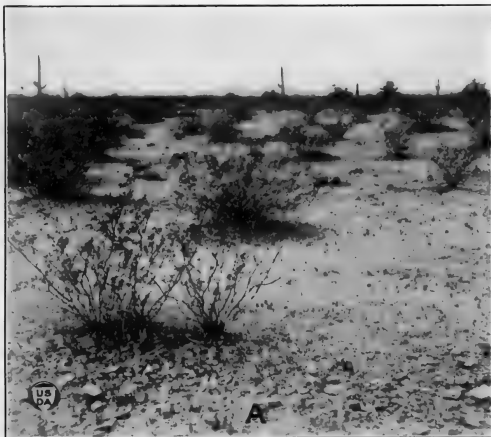
- A.-(60) Coleogyne. Near Bluff, Utah.**  
**B.-(61) Greasewood. San Luis Valley, Colo.**

**PLATE 11**

**A.-(65) Saltgrass. Seven miles north of Willard, N. Mex.**

**B.-(66) Pickleweed. Lund, Utah.**





**PLATE 12**

**A.-(69) Creosotebush. Sacaton, Ariz.**

**B.-(70) Creosotebush and bur-sage. Twenty-five miles east of Barstow, Calif.**

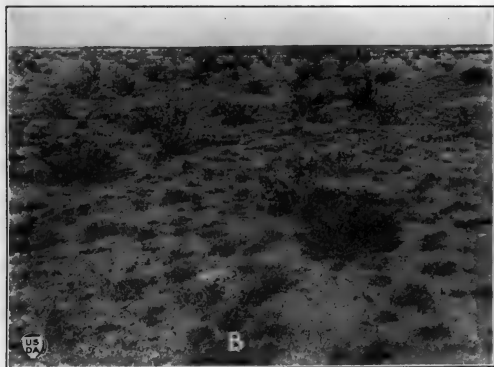


**PLATE 13**

**A.-(74) Creosotebush-desert grass. Six miles northeast of Hope, N. Mex.**

**B.-(78) Mesquite. Santa Rita Range Reserve, Ariz.**





**PLATE 14**

**A.-(84) Desert-sage. Thermal, Calif.**

**B.-(87) Black brush-grass land. West of Lakewood, N. Mex.**

**PLATE 15**

**A.-(89) Shinnery. Cheyenne, Okla.**

**B.-(91) Mountain brush land. Disappointment Canyon, Colo.**





PLATE 16

A.-(97) Chamise. San Diego, Calif.

B.-(98) *Ceanothus cuneatus*. Banning, Calif.



# THE DEVELOPMENT OF AMERICAN FOULBROOD IN RELATION TO THE METABOLISM OF ITS CAUSATIVE ORGANISM

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## INTRODUCTION

American foulbrood is one of the two serious diseases affecting the brood of the honeybee. The specific cause of this disease is a pathogenic, spore-forming microorganism, known as *Bacillus larvae*. The occurrence of this organism in uniformly pure culture, accompanied by the gross effects of its activity, as manifested by the characteristic appearance and age of the diseased and dead larvæ, differentiates American foulbrood from the other serious brood disease of bees, European foulbrood. The latter disease is caused by an entirely different non-spore-forming organism, *Bacillus pluton*, which causes a different manifestation of gross symptoms, complicated by the action of various secondary invaders.

Certain limited facts concerning the characteristics of the various types of bacteria concerned in causing or associated with these brood diseases have been studied, from which various practical applications have been derived. As has been stated by Phillips (39)<sup>1</sup>, "Bacteriological studies of bee diseases have been useful to practical beekeepers in explaining the reasons for success or failure with various treatments attempted. These studies have been especially important, however, because through them methods of laboratory diagnosis of the different diseases have been worked out."

Advancement in knowledge concerning the etiological and biochemical relationships of the brood diseases, particularly concerning differences in characteristics as related to gross symptoms, has been limited, however, because of the peculiar growth requirements of the causative organisms. There are fundamental differences between American foulbrood and European foulbrood, particularly as to characteristics of development, which, although recognized, have not been adequately explained by the incomplete data so far obtained on the metabolism of the causative organisms.

The present investigation was undertaken to obtain further data concerning the growth requirements of *Bacillus larvae*, the cause of American foulbrood, by which to explain these differences in the symptoms and development of the two diseases. Through improved methods of cultivation, a study has been made of factors concerned in the metabolism of *Bacillus larvae* correlated with certain hitherto unrecognized biochemical factors associated with the metabolism of the normal honeybee larva. The results obtained add materially to the knowledge of the biology of the brood diseases.

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Acknowledgments are due to Dr. E. R. Whitmore, professor of bacteriology and preventive medicine of the George Washington University, for much valuable advice and many suggestions, and to Dr. E. F. Phillips, apiculturist, Bureau of Entomology, United States Department of Agriculture, under whose direct supervision this work was done. Presented in part satisfaction of the requirements for the degree of doctor of philosophy at the George Washington University, April 21, 1923. This work was completed April 10, 1923.

<sup>1</sup> Reference is made by number (italic) to "Literature cited," p. 165-168.

## THE RELATION OF CONTRIBUTING CAUSES TO THE COMPARATIVE DEVELOPMENT OF THE TWO SERIOUS BROOD DISEASES OF BEES

In order to understand the basis upon which the consideration of this problem has been developed, it is necessary to make a comparative study of certain of the characteristics of the two brood diseases, American foulbrood and European foulbrood, aside from their etiology. It will be apparent from this study that certain contributing causes, although recognized and described, have not been further analyzed to any extent, particularly in relation to specific etiology. The experimental work of the present investigation is concerned primarily with American foulbrood, however, since the causative organism, *Bacillus larvae*, can be isolated and grown in pure culture, while as yet no artificial medium suitable for the growth of *Bacillus pluton*, the cause of European foulbrood, has been devised.

### RACE

It is an accepted fact that in American foulbrood the race or strain of bees has little or no relation to the development of or the resistance to the disease. This, aside from apparent lack of immunity or resistance of any of the races, may be explained partially by the fact that the decomposed material resulting from the death of the larvæ is of such a nature that the bees can not to any extent remove it from the combs after the disease has once become established. The dried-down masses (scales) are practically glued to the cell walls. *Bacillus larvae* forms resistant spores which allow the disease to be carried and spread almost indefinitely by means of the honey and old scales.

In European foulbrood, on the contrary, Italian bees seem to have some characteristic which makes them more resistant or vigorous in combating infection under the proper conditions. The results of bacterial decomposition of the diseased remains, even at their worst, are such that, if the colony is able to build up or is made sufficiently strong in worker bees, they are able to remove these remains, thereby removing the infection sufficiently to prevent its further development. *Bacillus pluton* does not form spores and lives only a comparatively short time under unfavorable conditions for growth, as in honey or on long drying. Furthermore, as has been demonstrated by the writer in a previous paper (45), this apparent resistance of the Italian bees was observed to be due largely to the racial characteristic of removing all foreign materials more promptly from the hive than do common black bees or hybrids, rather than to any natural resistance or immunity to the disease.

### STRENGTH OF COLONY

If a colony of bees has been exposed to infection from American foulbrood, the strength of the colony apparently has no direct relation to the development of the disease, except that strong colonies are usually the ones which rob the weaker infected colonies, thereby spreading the infection through the apiary. As suggested above, European foulbrood attacks primarily the weak colonies which have an insufficient force of bees to remove the infected material. Diseased combs from such a colony can be placed in a strong healthy colony of Italian bees with no resulting disease. This would be fatal in the case of American foulbrood.

### SEX

There has been slight mention in the literature of the relation of the sex of the bee larvæ to the development of disease. Phillips (38) states with regard to European foulbrood: "A symptom of greatest importance is the fact that the

disease attacks drone and queen larvæ nearly as quickly as those of the workers. The tendency of this disease to attack queen larvæ is a serious drawback in treatment. Frequently bees of a diseased colony attempt to supersede their queen but the larvæ in the queen cells often die, leaving the colony hopelessly queenless. The colony is thus depleted rapidly."

In American foulbrood, according to Phillips (39), "Usually the disease attacks only worker brood, but rare cases are found in which queen and drone brood are diseased." White (55) states, however: "That worker, drone, and queen larvæ are all susceptible to the disease has been demonstrated during these [White's] studies. Affected drone brood is encountered less often in the diagnosis of this disease than in that of European foulbrood. The writer has encountered queen larvæ affected by American foulbrood in experimental colonies only, although very probably diseased queen larvæ do occur in nature also." A few samples of diseased brood containing American foulbrood sent to the Bee Culture Laboratory for diagnosis have been found to contain affected drone larvæ as well as one or two cases of diseased queen larvæ. Although beekeepers believe that in American foulbrood drone brood is so seldom affected that the absence of diseased drone brood is a diagnostic character, the fact that occasionally drone larvæ do die of the disease makes it possible that some other factor than nonsusceptibility of sex is concerned. No accurate data are available on this subject. The work of this paper is concerned only with worker brood, because the great preponderance of worker brood affected gives slight importance to the comparatively few drone larvæ in the average colony.

#### AGE

The general characteristic difference in age between larvæ dying of American foulbrood and those dying of European foulbrood, mentioned at the beginning of this paper, has been one of the chief factors in the differentiation between the two diseases. Originally there was considered to be only one disease, "foulbrood." Although beekeepers have long known that brood of various ages is attacked by brood disease, it seems not to have been until about 1880 that the difference in age at the time of attack was used to separate foulbrood into two distinct forms, one "easily curable" and the other "virulent." Dzierzon (21) was the first thus to differentiate definitely into two types of disease, according to the difference in symptoms and age at time of attack. He stated that in the curable disease, "More of the larvæ die still unsealed, while they are still coiled in the bottom of the cell \* \* \*. The brood which does not die before sealing mostly attains to perfection \* \* \*. This is exactly the reverse in the malignant kind of foulbrood. In this the larvæ do not generally die before they have raised themselves from the bottom of the cell, have been sealed and begun to change into nymphs."

Cheshire (13) who probably was the first to investigate the bacteria associated with what, in the light of present knowledge, is known as European foulbrood, was inclined to agree at first with the distinctions made by Dzierzon. He soon stated (14), however, that Dzierzon was in error and that there is only the one disease, foulbrood, which he supposed was caused by an organism to which he gave the name *Bacillus alvei*. Cheshire and Cheyne (15) described *Bacillus alvei* as a spore-forming bacillus which they constantly found associated with a diseased condition of the brood and recognized only as "foulbrood." The results of this work caused considerable confusion to beekeepers and investigators, both in this country and abroad, for more than a decade.

In this country some time after 1890 it became evident to certain beekeepers, particularly in New York State, that they were dealing with two distinct diseases. The newly recognized form, which was found to attack the coiled larvæ,

was at first erroneously called "black brood," to distinguish it from the "foulbrood" of sealed larvæ. "Black brood" assumed epidemic proportions in New York State by 1897. This gave rise in American beekeeping literature to descriptions of two distinct diseases, as far as the age of the larvæ attacked and the appearance from the resulting decomposition were concerned.

#### RESULTING DETERMINATION OF ETIOLOGY

As a result of the increasing devastation by this new disease, work was started in New York State in 1902 (33), which was later carried on by White (49, 50), on the bacteriology of these brood diseases, by which doubt was cast upon *Bacillus alvei* being the cause of any disease, although it was found to be associated only with European foulbrood. Furthermore, a new spore-forming bacillus distinct from *Bacillus alvei* was observed and cultivated on special culture media from the disease attacking the sealed larvæ. This organism was at first designated *Bacillus X* but was later named *Bacillus larvae* (figs. 1 and 2). Subsequently this was found to be the cause of American foulbrood by experimental inoculation of healthy colonies with pure cultures (51). The symptoms

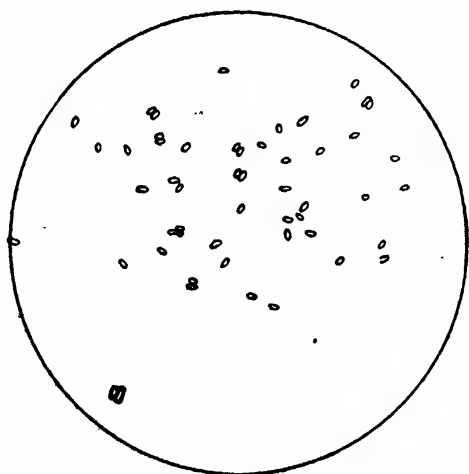


FIG. 1.—Spores of *Bacillus larvae*.  
(McCray (31))

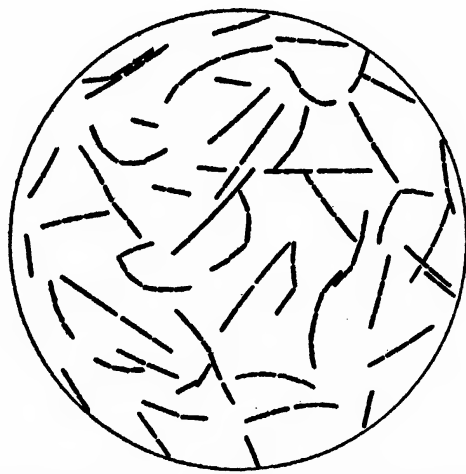


FIG. 2.—Vegetative rod form of *Bacillus larvae*.  
(White (55))

were accurately described and differentiated by Phillips (37), definite new names being used for the first time in order to eliminate confusion, as follows: American foulbrood, formerly known as "foulbrood" ("Usually the larvæ are attacked at about the time of capping, and most of the cells containing infected larvæ are capped"); and European foulbrood, originally called "black brood" ("This disease attacks the larvæ earlier than does American foulbrood, and a comparatively small percentage of diseased brood is ever capped").

Maassen (27) in Germany described at about the same time what is now accepted as the same organism as *Bacillus larvae*, a spore-forming organism constantly found to be present in the diseased brood dying after sealing, "Nymphen-seuche." He gave the name *Bacillus brandenburgiensis* to this organism. Burri (12) in Switzerland also recognized the fact that the spores present in large numbers in scales in the "nymph" disease were a new species that was difficult of cultivation.

White (52) later showed conclusively that *Bacillus alvei* is not the cause of European foulbrood but is only one of several secondary invaders. He demonstrated that the probable cause of European foulbrood is a nonspore-forming organism which he called *Bacillus pluton*. This organism develops before the

death of the larva in the intestinal tract and usually kills before sealing takes place, as differentiated from American foulbrood as described above. Unfortunately, as yet it has been impossible to grow this organism in pure culture on artificial culture media.

Further work has been done by various investigators on certain laboratory phases of the bacteriology and diagnosis of the two diseases, but no additional information has been obtained concerning the etiological and biochemical relationships of the causative organisms which would aid in the solution of the present problem.

#### BASIS FOR INVESTIGATIONS

Throughout all the discussion of symptoms of the brood diseases in the literature, particularly in relation to the different ages at which the diseases attack during the life history of the larvæ, there has been no adequate explanation of the reason for this apparent fundamental difference.

Maassen (28) in the case of American foulbrood made the observation that, "according to the microscopic findings from section preparations, *Bacillus brandenburgiensis* [*Bacillus larvæ*] does not come to luxuriant development in the intestine of the larva, though this is the case with *Bacillus alvei* and with *Streptococcus apis* [in 'sourbrood']. It finds much more promising nourishment in the fat bodies of the larva. Apparently the bacillus finds opportunity to press its way into the fat bodies shortly before the pupation of the bee, at the beginning of the natural changes in the intestinal tube. From this it seems clear why the larvæ containing *Bacillus brandenburgiensis* die after sealing." In part this is probably correct, since it may easily be observed that soon after capping the tissues of the healthy larva become more or less granular and watery in consistency, at which time it is almost impossible to distinguish the intestinal tract. It is also difficult to remove the larva in this condition from the cell without rupturing the skin envelope. This process is described more in detail later. It does not explain, however, why the spores of *Bacillus larvæ* do not germinate and increase in numbers sufficiently to kill the larva much earlier during the feeding period, as in the case of European foulbrood. A vague and only partially correct suggestion was given in an earlier paper by the writer (46), in which the following theory was stated: "*Bacillus larvæ* gains entrance to the larva generally in the spore stage, in the larval food. This occurs at about the same stage as in European foulbrood, while the larva is still coiled in the cell. Only rarely, however, do coiled larvæ die. This is apparently because it takes some time for the resting stage spores to germinate into the active vegetative rods. This causes death, as a rule, to occur later in the life history of the larva."

#### RELATION OF THE BROOD DISEASES TO THE LIFE HISTORY OF THE HONEYBEE LARVA

The development of the honeybee may be divided in general as follows: After the egg is laid there is a period of three day's incubation before it hatches into the larva. The larval stage, during which active feeding and growth occur, comprises four and a half to five and a half or six days. At the end of the feeding period the larva is sealed in the cell, where it spins its cocoon. Metamorphosis then occurs, and the fully formed adult bee emerges in about 12 days, making a complete developmental period of approximately 21 days. According to White (53), there is a prepupal period in healthy brood of four days after sealing occurs before the actual change in the external form to that of the adult bee takes place. During the first two days after capping, the larva is active in the cell, consuming any remaining food and spinning a cocoon. Some time during this period according to Straus (43), or just previous to capping according to Zander (57),

the larval intestine, which up to this time has been a blind sac, is connected with the end gut, allowing defecation to take place. There is then two days of quiescence, during which the larva extends in the cell and lies motionless, while internal changes preparatory to metamorphosis occur (figs. 3 and 4). These changes (?) consist of the almost complete histolysis of the fat body of the larva in order to furnish nutriment for the formation of imaginal tissues. This is made possible by the physiological and morphological changes occurring in this stage of the development of the larva. Extended investigations have been made of these physiological and morphological changes, but they need not be summarized further here, since the present work has been solely of a biochemical character. It is noticeable, however, that the intestines of mature larvæ even for a short time after capping are full of material colored by the pollen content, while the intestines of the prepupæ, after they have extended in the cell, are colorless.



FIG. 3.—Healthy prepupa approximately 8 days old, having reached the quiescent stage. This is the age at which the majority of larvæ die from American foulbrood. End view. (White (55))

It is during the latter two-day prepupal period that according to Maassen (28) the invasion of the fat body by *Bacillus larvae* occurs and that according to White (55) the majority of the brood dies in American foulbrood.

In European foulbrood, on the contrary, the majority of the larvæ in typical cases of this disease die before sealing and after reaching an age of  $3\frac{1}{2}$  to 4 days from the time of hatching of the egg (56) (fig. 5). In certain

abnormal cases in European foulbrood death may occur after capping (46), but this almost always occurs during the first two days of the prepupal stage, when the larva in most cases is still moving about in the cell, usually causing a gross appearance quite different from that of brood dead of American foulbrood.

#### PRELIMINARY EXPERIMENTS

While studying the bacterial flora associated with the early stages of European foulbrood in the larval intestine certain results were obtained which suggested a possible explanation of the delayed development in American foulbrood. Until death takes place in European foulbrood the growth of the organism causing the disease and certain secondary associated forms occur only within the intestine (52); that is, within the peritrophic membrane, but not in actual contact with living tissues of the larva. It is only after death that the secondary invaders, particularly *Bacillus alvei*, invade the body tissues (45).

Another important distinction which must be considered is that the feeding of the larva is not the same throughout larval life. Von Planta (40) has shown that for the first part of the feeding period one type of food is used by the larva and that at a later stage a food different in chemical and physical composition is provided. Young larvæ receive a food for a time after hatching that is much richer in fat and albuminous material but lower in sugar content than that fed to older larvæ. The food of the older larvæ, which is known to consist mainly of honey or nectar and pollen, is much higher in sugar content, while there is a considerable decrease in fat and albuminous material. The sugar in the food of the older larvæ, particularly

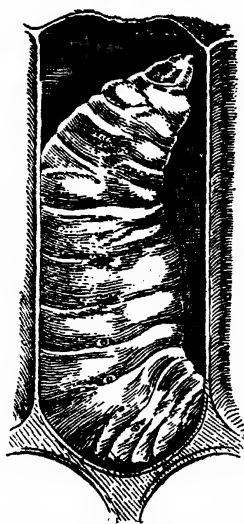


FIG. 4.—Healthy prepupa Side view. (White (55))

that of larvæ at the age when European foulbrood makes its attack, comprises nearly 45 per cent of the dried substance, or nearly 14 per cent of the fresh substance. From these facts it may be assumed that, because of the great amount of food given the larva at this age, there must be present, in the larval intestine, at all times during the active feeding period, considerable amounts of this food rich in sugar unassimilated, up to and even after active feeding ceases. A number of larval and prepupal intestines were dissected from healthy larvæ and tested roughly with Benedict's qualitative solution (34) for the presence of reducing sugars. The results indicated the presence of relatively large amounts of reducing sugar in the intestines of larvæ just prior to sealing. Little or no reducing sugar could be demonstrated in the intestines of sealed larvæ or prepupæ.

It may therefore be assumed that certain of the organisms associated with the early stages of European foulbrood are able to grow in the presence of a high sugar concentration. Experiments were devised in which a medium containing 10 per cent dextrose was used. It was found that while a few types of organisms, such as one resembling *Streptococcus apis* (28), could be grown in varying numbers, an organism similar to that described by Maassen (29), resembling the larger forms of *Bacillus pluton*, called *Bacillus lanceolatus*, could be isolated and grown from over 50 per cent of the samples cultured. As described by the writer (47), "This organism was found to grow best on a 10 per cent dextrose yeast extract agar with a reaction slightly acid. It is differentiated from *Bacillus pluton* and *Streptococcus apis* in being gram-negative, and does not grow readily if at all in media without sugar." From these studies it was suggested that possibly this comparatively high sugar content of the unassimilated food in the larval intestine may have an influence on the germination of the spores and growth of *Bacillus larvae* and that a change may occur when the sugar content is sufficiently reduced by assimilation in the larval intestine after it has been capped and when the intestines have been emptied by the opening of the ventriculus into the end gut. Therefore, with these preliminary observations as a basis, experimental work on this subject was begun during the spring of 1922.

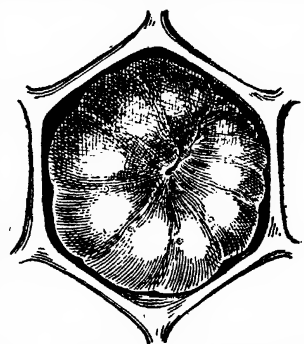


FIG. 5.—Healthy coiled larva at age of maximum intestinal sugar content and approximately the age when the majority die from European foulbrood. (White (58))

## GROWTH OF BACILLUS LARVAE IN CULTURES IN RELATION TO VARIATION IN SUGAR CONCENTRATION

The first step in the substantiation of this theoretical assumption is to determine whether there is a correlation between germination of the spores of *Bacillus larvae* and vigor of vegetative growth and variations in concentration of reducing sugars in culture media. Ordinary culture media are unsuitable for the growth and isolation of *Bacillus larvae*; in fact, one of the confirmatory tests for this organism in laboratory diagnosis of American foulbrood (31) is the absence of growth on plain beef infusion agar plates, since the spores will not germinate thereon. There are rarely any secondary invaders associated with *Bacillus larvae* in the decayed material, and these plates practically never show growth.

### GROWTH REQUIREMENTS OF BACILLUS LARVAE

Various special culture media have been devised which answer more or less satisfactorily the requirements for the ordinary growth of the organism. The

spores of *Bacillus larvae* will germinate and grow feebly on an agar medium in the preparation of which healthy bee larvæ are used as is meat in ordinary culture media, sterilizing as usual by heat in an autoclave (49). However (51), if a broth made by macerating healthy bee larvæ in several times their volume of water is sterilized without heating by filtering through sterile bacteria-proof filters and then is pipetted aseptically into tubes of previously sterilized liquefied agar cooled to 50° C., the resulting medium gives much better growth. This medium is nevertheless unsatisfactory, owing to difficulties of preparation, and particularly because of lack of material for its preparation except during the brood-rearing season. White (54) therefore devised a medium which consists of a suspension of the yolk of an egg aseptically in 70 cc. of sterile water, 1 cc. of which suspension is added by sterile pipette to each 5 cc. of ordinary sterilized tubed agar medium which has been melted and cooled to 50° C. Growth occurs on this medium quite abundantly, although with the technic described great care must be taken to prevent contamination.

Maassen (28) has also devised a medium made from a mixture of equal parts of a broth from calf or pig brain and a solution of egg albumin in water, to which 1.8 per cent agar and 1 per cent each of Witte's and Chapoteaut's peptone are added, after which it is filtered, tubed, and sterilized. This medium gives an almost neutral or weakly acid reaction to blue litmus paper. Maassen also found that the vegetative forms develop abundantly if grown on a meat and water medium if it is acid in reaction and if 0.25 per cent of pollen and 1.5 per cent of Aschmann's or Chapoteaut's peptone are added, but that the former medium is more favorable. Both media are found to deteriorate on too much heating. It is also stated that in acid peptone bouillon, in bouillon of bee larvæ, and in the brain bouillon, the bacillus may be cultivated, although growth is slow, the bouillon becoming weakly turbid and a thick slimy deposit gradually being formed.

For the purpose of the present experiments, after consideration of the advantages or disadvantages of the various media so far described, a modification of the egg-yolk suspension medium of White was adopted as the most satisfactory general medium. During the course of the experiments some modifications were made both in the medium and in the technic of preparation.

#### PREPARATION OF YEAST-EXTRACT AGAR BASE

Because of most satisfactory results in other work with various brood disease cultures, a yeast-extract agar described by Ayers and Rupp (2) was used instead of beef infusion agar as a base, because of the ease of preparation and the uniformity of the medium. Spores of *Bacillus larvae* on the surface of a slant of this agar germinate to some extent on this medium alone, and vegetative cultures from egg-yolk suspension agar transferred to the yeast medium grow fairly vigorously. The addition of egg-yolk suspension to the yeast-extract agar increased the vigor of growth and longevity of cultures.

One liter of the yeast extract agar is prepared as follows:

Dried yeast.....	grams..	10
Peptone.....	do.....	10
Buffer (sodium glycono-phosphate).....	do.....	5
Water.....	cc.....	500

This is heated in flowing steam for one-half hour, then adjusted to a hydrogen-ion concentration of  $P_H = 7.6$  to 7.8 by the colorimetric method of Clark and Lubs (16, 17). The broth is then boiled for one minute over an open flame and filtered through filter paper on a perforated porcelain funnel, using siliceous earth to clarify. To this broth is added an equal amount (500 cc.) of double strength



(3 per cent) solution of agar, washed and filtered by the method described by Ayers, Mudge, and Rupp (3). The final hydrogen-ion concentration reaction is adjusted so that upon addition of 1 cc. of the egg-yolk suspension to 10 cc. of the yeast-extract agar the reaction is about  $P_{\text{H}}=6.8$ . The normal hydrogen-ion concentration value of the contents of the larval intestine at various ages during the active feeding period with honey and pollen and just after sealing averages  $P_{\text{H}}=6.8$ , varying to slightly more acid with the amount and type of pollen in the food material. Intestines were dissected out from the larvæ and macerated in 10 cc. of neutral distilled water and compared colorimetrically with known buffer solutions, using brom thymol blue as an indicator. Fabian and Parks (22) found this value to be  $P_{\text{H}}=6.6$  by macerating the entire larva in water. From earlier unpublished work by the writer, as well as by the above-mentioned investigators, the optimum hydrogen-ion concentration for the growth of *Bacillus larvæ* was found to be approximately  $P_{\text{H}}=6.8$ . The yeast extract medium is tubed, sterilized in the autoclave at 15 pounds pressure for 15 minutes, and stored until needed.

#### PREPARATION OF EGG-YOLK SUSPENSION

The egg yolk can be diluted much more than was directed in the original formula with even better results, the more dilute suspension giving a more transparent medium with fully as profuse growth. A wide-mouthed flask containing 200 cc. water, sterilized with a cotton plug protected by a paper cap, is used for each egg yolk. At times, from 0.5 per cent to 1 per cent of a neutral buffer salt is added to the water previous to sterilization. This holds in check the slow increase in acidity observed on long standing. A small amount of normal sodium hydroxid (2 to 3 cc.) is also added to the flasks before sterilization to bring the resulting reaction of the egg suspension nearer to the desired reaction for the final medium.

#### APPARATUS TO REPLACE PIPETTING

An apparatus was devised (fig. 6) which to a great extent eliminates the danger of contamination of agar tubes when adding egg-yolk suspension, and also makes possible the preparation of a large quantity of medium in a short time. As a rule egg-yolk suspension can be stored or withdrawn at any time after the apparatus has been set up, until all used up, unless the egg yolk itself is not sterile. A two-holed rubber stopper, of correct size to fit the flask containing the egg-yolk suspension, is fitted with two tubes, one of small bore to reach nearly to the surface of the liquid when placed in the egg-yolk flask, and a second larger tube fitted flush to the inner surface of the stopper, protruding outward about  $1\frac{1}{2}$  inches. A piece of rubber tubing 5 inches long is fitted to this tube, closed with a pinchcock. To this rubber tube is attached a delivery tube which passes through another rubber stopper placed in one end of a glass cylinder  $1\frac{1}{2}$  inches in diameter and 4 inches long, to about half its length. This forms a protective bell for the delivery tube similar to that used in filling vaccine or antitoxin ampules. The

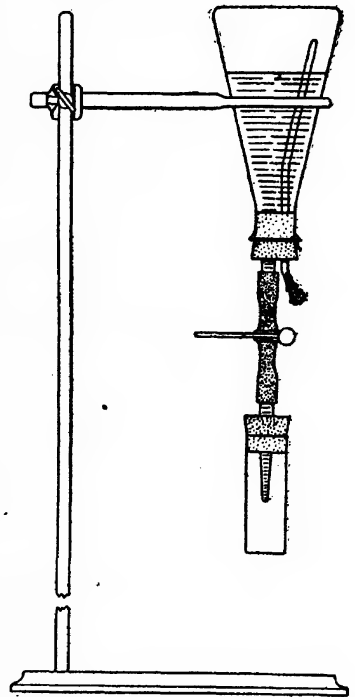


FIG 6.—Apparatus to replace pipetting of egg-yolk suspension

entire apparatus is sterilized in the autoclave, using a temporary empty flask into which the stopper for the culture flask is placed, and all is wrapped in paper with a paper protective cap over the open end of the delivery bell. Before use, the apparatus is removed from the paper and the stopper is carefully removed from the empty flask so as to prevent contamination and is fastened firmly in the flask containing the egg-yolk suspension. After placing the pinch cock in position, the apparatus is carefully inverted and hung on a ring stand. The small-bore glass tube in the flask now reaches a little above the surface of the liquid and serves for an air inlet. By means of this apparatus, sterile egg-yolk suspension can be added to tubes of sterile base medium, with little danger of external contamination, by inserting the tube under the protective bell.

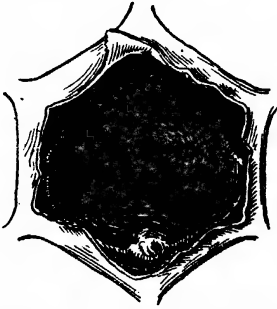


FIG. 7.—American foulbrood scale. End view. (White (55))

which from 1 to 2 cc. of egg-yolk suspension is added for each 10 cc. of base, by means of the apparatus described above. The contents of the tubes are well mixed and then slanted.

From a comb containing decaying material dead of the disease, a dried scale (figs. 7 and 8) is removed with a sterilized needle scalpel (also used for removing cappings) and dropped into the water of condensation in the culture tube to soften. It is then smeared over the surface of the agar with an inoculating needle. If ropy gluelike material is available it is more satisfactory (fig. 9). A large loopful of this is removed from the cell, from which the capping has been aseptically removed by means of an inoculating needle, and is streaked over the surface of the agar. A heavy initial inoculum gives best results, as it is often difficult to obtain growth with a small amount. It is quite easy to obtain pure cultures by this procedure, since almost never are secondary contaminations found associated with *Bacillus larvae*. Plating may be carried out from these initial cultures if absolute surety is desired, but initial growth is obtained much more easily by the tube culture method. Germination of spores and some growth take place during the first 24 hours' incubation at 37° C., but maximum growth is not obtained much before 48 hours.

#### EXPERIMENTAL PROCEDURE, USING AGAR SLANTS

To determine whether there is a correlation between germination of spores and vegetative growth of *Bacillus larvae* and the concentration of sugar in the culture medium, a series of tubes is prepared with varying percentages of dextrose, from 0.5 per cent to 10 per cent (Table I). These are prepared by adding the required amounts of dextrose to 50 cc. portions of the yeast-extract agar base, which is then tubed and sterilized at 10 pounds pressure for 15 minutes. On cooling to 55° C., 1 cc. of sterile egg-yolk suspension is

#### METHOD OF ISOLATION OF PURE CULTURES OF *BACILLUS LARVAE*

When medium is desired for the isolation or cultivation of *Bacillus larvae*, tubes of the yeast-extract agar are melted in a water bath and cooled to 55° C., after

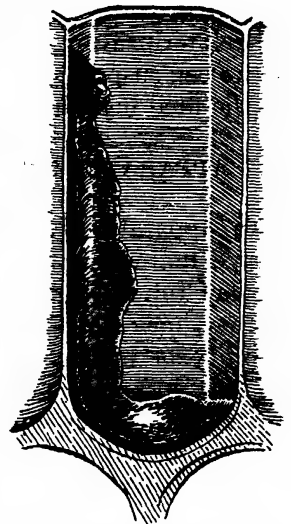


FIG. 8.—American foulbrood scale. Side view. (White (55))

added to each tube and it is then slanted. Series of agar slants varying in sugar concentration are inoculated with either vegetative cultures or diseased material containing only spores. To determine spore germination an approximately uniform amount, about one 2-mm. loopful of ropy material, when available, is used for inoculation of slants, otherwise a scale softened as described above. If no visible growth takes place after 48 hours' incubation, stained smears are made, to determine whether any germination has occurred. In the case of the determination of growth from vegetative culture, a single uniform streak is made on the agar slant, using one 2-mm. loopful of growth from a 48-hour culture of *Bacillus larvae* previously isolated and cultivated. After 48 hours' incubation, as well as after about one week, comparative observations are made of the relative amount and character of the growth. Where little or no growth has occurred, stained smears are made from the streak to see what has happened to the organisms. These experiments were carried out with a number of different strains of vegetative cultures and from a number of different samples of American foulbrood.

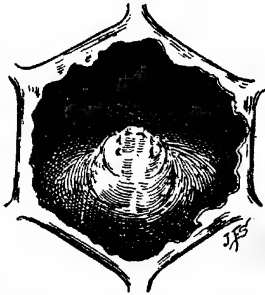


FIG. 9.—Partially decomposed American foulbrood larva at the stage of ropy consistency. (White (55))

TABLE I.—The effect of varying the sugar concentration in egg-yolk suspension medium (1) on germination and vegetative growth from spores; and (2) on vegetative growth from vigorous vegetative cultures of *Bacillus larvae*.<sup>a</sup>

Test material	Per cent dextrose in medium							
	Control	0.5	0.7	1.0	1.3	1.5	1.75	2.0
Spores.....	++++	++	+++	++++	++++	+++	+++	++
Vegetative cultures.....	++++	+++	+++	++++	++++	++++	+++	+++

Test material	Per cent dextrose in medium									
	2.25	2.5	2.75	3.0	3.5	4.0	4.5	5.0	7.5	10.0
Spores.....	+	+	+	+	—	—	—	—	—	—
Vegetative cultures.....	++	++	++	++	+	+	±	—	—	—

<sup>a</sup> The following symbols are used:  
+ Slight growth.  
++ Fair growth.  
+++ Good growth.  
++++ Heavy growth.

± Doubtful.  
— No evidence of growth.  
G Slight germination of spores.

EXPERIMENTAL PROCEDURE, USING PLATE CULTURES

The egg-yolk suspension agar is not entirely satisfactory for counting colonies in plate cultures, since the egg yolk gives the medium a cloudy, semiopaque appearance. However, by using the supernatant fluid from the egg-yolk suspension or a somewhat smaller amount of the suspension for each tube of yeast extract agar (10 to 15 drops), a fairly satisfactory plate culture is obtained if the proper amount of inoculum is used. The following procedure is used: To a series of melted tubes of yeast-extract agar containing varying amounts of dextrose as described above (Table II) the egg-yolk suspension is added and the desired inoculation of the tube made while the medium is still liquid. The tubes are agitated to mix the contents thoroughly and then poured into sterile Petri dishes.

These on cooling are inverted and incubated for 48 hours at 37° C., after which counts are made. If the plates are flooded with a dilute solution of fuchsin or eosin before counting, the colonies are more easily differentiated for counting in the semiopaque medium. Vegetative cultures only were used for plating. A suspension of one loopful of culture in 3 cc. of sterile broth is made and one loopful of that is used to inoculate each plate. Dilution in sterile water was also tried, using 1 cc. of the dilution for each plate, but without success, since there seems to be a minimum amount of initial inoculum required, below which it is difficult to obtain growth.

TABLE II.—Average number of colonies per 4-mm. loopful of vegetative culture suspension in broth on plates of varying sugar concentration

Per cent dextrose	Average number of colonies	Per cent dextrose	Average number of colonies
Control (o) -----	1, 500	2. 0 -----	-----
0. 5 -----	1, 590	2. 5 -----	150
1. 0 -----	1, 560	3. 0 -----	0
1. 5 -----	914	3. 5 -----	0

OBSERVATIONS

SPORE GERMINATION AND GROWTH IN RELATION TO SUGAR CONCENTRATION

At different times during the investigation seven different series of culture tubes were made, using as material for inoculation either scales or, in most cases, ropy remains heavily laden with spores of *Bacillus larvae*, but no vegetative rods. This material was taken from six different samples of diseased brood from different localities. From these series of cultures, varying in sugar concentration from 0.5 per cent to 10 per cent dextrose, it was found that active growth occurs up to and including 2.5 per cent dextrose, although some growth occurs occasionally up to 3 per cent (Table I). The exact limits varied slightly with different strains as well as with variation in the amount of inoculum. Even up to 10 per cent dextrose concentration, a varying small number of spores germinate, as is demonstrated by stained smears, but they give no further evidence of vegetative growth upon the culture medium.

GROWTH FROM ACTIVE VEGETATIVE CULTURES

In a similar manner five different series of tubes with varying sugar concentrations were made, using 24-hour cultures of three different characteristic vegetative cultures of *Bacillus larvae*, previously isolated and accustomed to growth on artificial culture media for different lengths of time. Good growth occurs on the average up to 2.5 per cent to 3 per cent dextrose concentration, with evidence of varying slight growth up to 4 per cent and in one case up to 4.5 per cent (Table I). In the latter case much of the variation is due to variation in the amount of initial inoculum. If a heavy inoculation is made on the surface of the agar tubes, the upper sugar concentration limits for inhibition of growth are increased, although in these cases the growth was meager at best. Stained smears, however, made after a few days, from the higher sugar concentrations particularly, soon showed the peculiar disintegration of the rods noted by White (55) as taking place in old cultures and where spore formation is inhibited, such as in the presence of sugar. This, according to observations of Sturges and Rettger (44) on other organisms, suggests that this disintegration of the rods is the result of autolysis.

QUANTITATIVE GROWTH IN PLATE CULTURES

Great difficulty is found in obtaining satisfactory plate cultures. Only two series of plate cultures were obtained which could be counted successfully. The average number of colonies showed a definite decrease with increased sugar concentration, with no growth at 3 per cent or higher. (Table II, fig. 10). As stated above, the plate method, with the small amount of initial inoculum necessary for accurate counts, is not a satisfactory method for obtaining growth of *Bacillus larvae* under these conditions, although the method may be used for obtaining pure cultures.

From these observations (Table I) it is, therefore, safe to conclude that a concentration of reducing sugar of approximately 3 to 4 per cent or more inhibits the growth of *Bacillus larvae*, although slight germination of spores may take place at higher sugar concentrations.

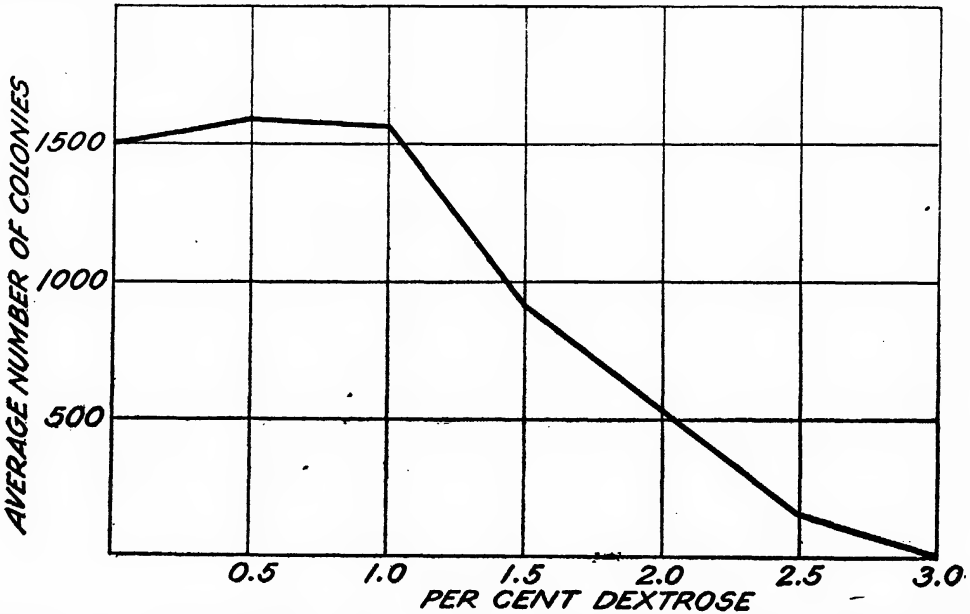


FIG. 10.—Average number of colonies per 4-mm. loopful of vegetative culture suspension with varying sugar concentration (Table II)

QUANTITATIVE DETERMINATION OF UNASSIMILATED SUGAR IN THE LARVAL INTESTINE AT VARIOUS AGE PERIODS

In the preliminary experiments it was shown that unassimilated sugar is present in the intestinal contents of the actively feeding larva, which apparently is assimilated completely by the time the prepupa has extended in the cell and has become quiescent. Since it is demonstrated that a direct relation exists between the growth of *Bacillus larvae* in suitable culture media and its reducing sugar concentration, it is now necessary to determine quantitatively the amount of unassimilated sugar in the intestine of the feeding larva and in the intestine of the prepupa, in order to determine whether reducing sugar concentration has any bearing on the time of attack by American foulbrood.

COMPOSITION OF BROOD FOOD

The older bee larvæ (40) receive a food consisting of a mixture of honey or nectar and pollen, rich in sugar, chiefly reducing sugar. This sugar constitutes about 45 per cent of the dried substance, or over 13 per cent of the fresh substance. The food of younger larvæ contains only about 5 per cent of sugar in the fresh

material (Table III, fig. 11). Nelson and Sturtevant (35) and Lineburg (26) have shown that the change in the composition of this food comes definitely soon after the second day, instead of the fourth day, as stated by Von Planta, after which increasingly large amounts of honey and pollen are fed up until the time of sealing. The larva is fed during this period about as fast as it can ingest the food. From this it is reasonable to suppose that there must be a constant surplus of unassimilated food in the larval intestine until after feeding has ceased.

TABLE III.—Percentage composition of worker brood food, calculated from Von Planta (40), and on the basis of his assumption of 70 per cent water content

Substance	Under four days		Over four days	
	Dried substance	Fresh substance	Dried substance	Fresh substance
	Per cent	Per cent	Per cent	Per cent
Nitrogenous.....	53.38	16.01	27.87	8.36
Fat.....	8.38	2.51	3.69	1.11
Glucose.....	18.09	5.43	44.93	13.48

COMPOSITION OF HONEY

The average chemical analysis of American honeys has been shown by Browne (9) to be as follows: Moisture 17.59 per cent, invert sugar 74.41 per cent, sucrose 1.98 per cent, ash 0.23 per cent, dextrin 2.09 per cent, undetermined 3.70 per cent. Approximately the same percentages have been found by all other workers in this field. The maximum sucrose content of honey is given in American standards for food analysis as 8 per cent, although a few samples have been found with a slightly higher sucrose content. In the utilization of honey as food by either the adult bee or the larva, it may be assumed that sucrose is rapidly hydrolyzed. In any analysis of the stomach content of the bee larva for sugar content, therefore, after the change in larval food has occurred and when honey enters directly into its composition, it may safely be assumed that a determination of the amount of reducing sugar will indicate the amount of unassimilated sugar in the intestine, since there will be but a small additional sugar content from sucrose, if any of the latter sugar still remains. In determining the sugar content of the whole larva, as was done in most of the present work, it may be assumed that there is a comparatively small amount of reducing sugar in the blood stream, because of the exceedingly rapid transformation of these sugars into fat and glycogen which are known to occur in the bee larva. It is therefore concluded that the sugar found in the whole larva is virtually that which occurs in the intestine alone, and this greatly simplifies the work of analysis.

COMPOSITION OF THE LARVA AT DIFFERENT AGE PERIODS

The work of Straus (43) on the chemical composition of the worker and drone brood during their different developmental stages gives the results of the metabolism of this food, as indicated by the presence of fat and glycogen stored in the so-called fat body of the larva (Table IV, fig. 12). He was unable to demonstrate more than a trace of what he terms reducing substances, except in one case in which only a slight amount was found. He believes that this is because the sugar of the larval food is assimilated so rapidly, as is indicated in the larval composition by the exceedingly rapid increase in the amount of glycogen and fat until after feeding has ceased.

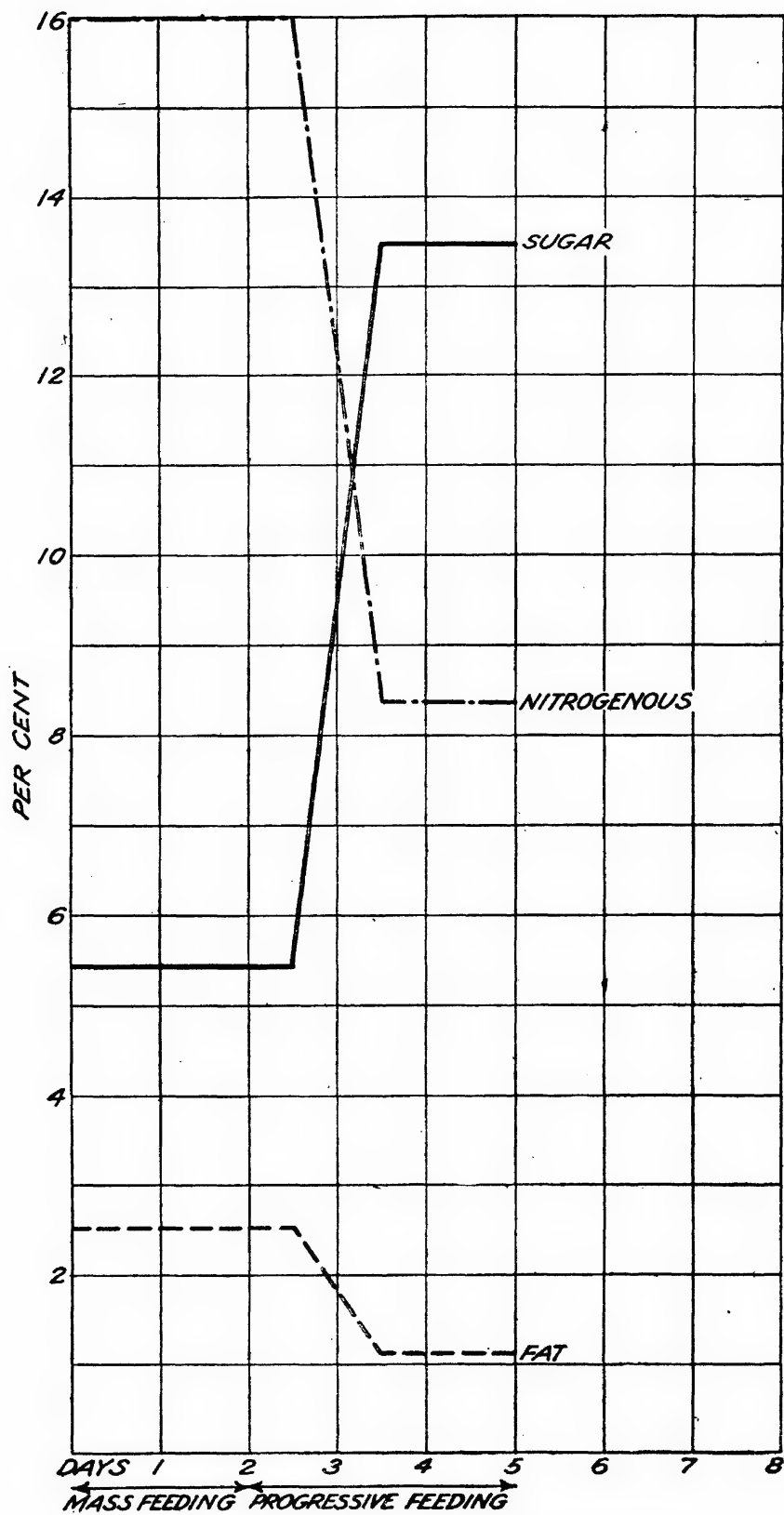


FIG. 11.—Per cent composition of worker brood food (Table III)

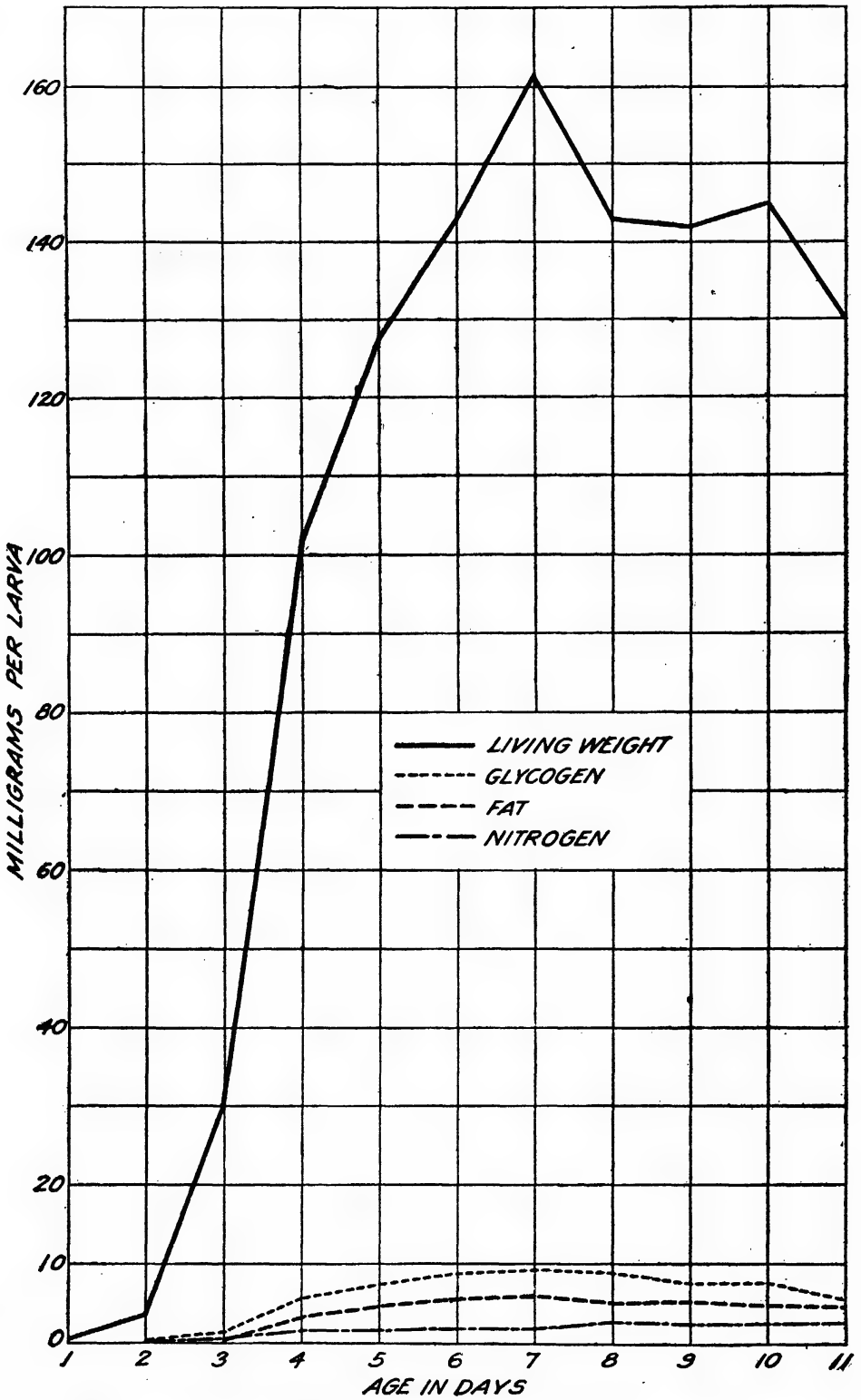


FIG. 12.—Average chemical composition of worker larvæ at different ages (Table IV)



TABLE IV.—Average chemical composition of worker larvæ at different ages, compiled from Straus (43)

Age	Weight of larva	Glycogen		Fat		Nitrogen		Reducing sugar
		Grams per larva	Per cent of fresh substance	Grams per larva	Per cent of fresh substance	Grams per larva	Per cent of fresh substance	
Days	Grams							
1.....	0.00030							
2.....	.00340	0.00008	2.50	0.00004	1.53	0.00009	2.86	
3.....	.03000	.0012	2.75	.00005	1.64	.0005	2.04	
4.....	.10010	.0055	5.58	.0031	3.60	.0016	1.44	
5.....	.12775	.0072	5.57	.0047	3.64	.0016	1.47	0
6.....	.14290	.0088	6.95	a .0057	a 3.98	.0019	1.45	
7.....	.16140	.0092	6.43	.0060	3.71	.0018	1.22	Trace.
8.....	.14300	.0089	6.35	.0051	3.53	.0027	1.51	Trace.
9.....	.14200	.0075	5.21	.0052	3.66	.0022	1.60	
10.....	.14500	.0075	5.24	.0049	3.60	.0022	1.58	0.0002
11.....	.13000	.0056	4.21	.0047	3.26	.0023	1.68	

a Calculated by interpolation and averaging.

CHOICE OF REAGENT

It was necessary to devise a special technic for the determination of the unassimilated reducing sugar in the larva by the application of procedures used in other analyses where small amounts of reducing sugars must be determined, such as in urine analysis. After studying the various methods of sugar analysis, a volumetric titration method seemed the most promising.

For the purpose of determining quantitatively the unassimilated sugar in the bee larva at different ages, the modified copper sulphate solution of Benedict (5) was chosen, mainly because, as in urine analysis, it has proved more satisfactory than any other titration method for determining small amounts of reducing sugars quantitatively, and because this solution keeps indefinitely without deteriorating. The potassium sulphocyanate in the solution produces, upon reduction of the sugar, a white precipitate of cuprous sulphocyanate, which permits the end point of the reaction to be more accurately determined than with Fehling's solution. A trace of ferrocyanid is added to prevent precipitation of red cuprous oxid which may be caused by certain impurities, which would interfere with the determination of the end point. The test solution is standardized to a known solution of dextrose so that 5 cc. equals 0.0102 grams of dextrose.

CHOICE OF LARVAE

Since there is little likelihood of there being any appreciable amount of sugar elsewhere than in the intestine, analyses were made of entire larvæ, because of the great difficulty attending the dissection of the intestines. Larvæ for analysis were chosen from combs having large areas of brood of uniform size and age. In most cases 25 larvæ as nearly of the same size as possible were carefully removed from the cells by means of a pair of fine forceps, care being taken to remove as little uningested food as possible. Any visible amount of adhering food was removed with filter paper and the 25 larvæ were weighed. Several series were weighed for each age above the two-day age period through to about the fourth day after capping.

DETERMINATION OF AGE OF LARVAE

When choosing larvæ for the analysis, the approximate age was determined by comparison with drawings to scale by Nelson and Sturtevant (35) of larvæ of known age at various age periods, 24 hours apart. Nelson and Sturtevant, as

well as Straus (Table IV), also give weights for larvæ of known age, but in order to eliminate the danger of variations due to the effect of different seasonal and environmental conditions, the average age of the larvæ analyzed from various groups of 25 was determined by comparison with a series of weighings of larvæ of known age that were made during this same period (35). The various series of weights, with the corresponding determinations of reducing sugar, were arranged in age groups, 24 hours apart, as shown in Table V. In some cases, such as the small two-day larvæ, or the quiescent prepupæ, where the amount of unassimilated sugar is small, 50 larvæ were taken for analysis, but usually 25 proved satisfactory.

#### PREPARATION OF MATERIAL FOR ANALYSIS

Several difficulties were encountered in the preparation of material for sugar determination. At first, attempts to extract the sugar were made by macerating the larvæ with distilled water and filtering through filter paper. This produced a cloudy opalescent liquid, indicating the presence of colloidal material, and this solution did not give the characteristic reaction with the Benedict reagent. Various clarification methods were tried. Precipitation with both neutral and basic lead acetate (10, p. 276) solutions proved unsatisfactory, something still remaining to interfere with the reaction. Mercuric nitrate solution, which is sometimes used to clarify liquids of animal origin such as blood, urine, and milk, was tried (10, p. 447). This method occasionally gave good results, mainly with the younger larvæ, but often with older larvæ and prepupæ the colloidlike material still remained in the filtrate, interfering with the reaction. Furthermore, because of the numerous filtrations necessary to remove successive precipitates, it was feared that more or less sugar is lost by adsorption to those precipitates, even with careful washing. An attempt was made to clarify by filtration with suction through a celloidin membrane, and this gave a clear solution which reacted well with the test solution, but the method required too great time. The method finally adopted was by extraction with 50 per cent alcohol, similar to the method used in the extraction of sugars from grains and similar products (11). This method proved successful, since the alcohol causes precipitation of all solid matter, giving a clear filtrate which reacted properly with the Benedict's reagent. Since glycogen in water solution is colloidal in nature, and thereby difficult to remove by filtration from such a solution, it is doubtless the glycogen present in the larva which prevented clarification and interfered with the reaction. It is possible for this reason that Straus (43) failed to demonstrate reducing sugars. To determine this point, a small amount of glycogen was added to a known solution of dextrose and tested with the copper sulphate solution, and the known reducing sugars could not now be demonstrated quantitatively. Since glycogen is insoluble in alcohol (10, p. 443) the 50 per cent alcohol precipitates the glycogen and thereby removes materials interfering with the reaction in the filtrate. Even though there may still be a small loss of reducing sugar by adsorption or by some other means, the results obtained are of value for purposes of comparison. If any reducing sugar is lost by the method adopted, the amount is exceedingly small and may therefore be disregarded, since repeated washings failed to demonstrate its presence.

#### TECHNIC ADOPTED

After weighing, the larvæ are removed to a small porcelain mortar and macerated in 30 cc. of 50 per cent alcohol. This material is then washed carefully into a small flask and allowed to stand from two to three hours before filtering. The precipitate is washed with 50 per cent alcohol. The filtrate is then made up to 50 cc. with distilled water, and run into a burette. Five cc. of the stand-

ardized Benedict's solution are placed in a white porcelain casserole and diluted with an equal amount of distilled water. To this are added about 5 grams of anhydrous sodium carbonate and a small amount of ground pumice. This solution is brought to a boil and the larval extract is run in slowly, drop by drop at the end, until the blue color disappears and a white precipitate forms. From the number of cc. of larval extract used, the milligrams of sugar per larva and the per cent of sugar per larva are calculated (Table V).

TABLE V.—Unassimilated sugar in intestinal content of larvæ at different ages

Larvæ of known age, Sturtevant (55)			Larvæ analyzed for presence of unassimilated sugar (weights in grams)									
Age in days	Average weight	Limits by weight for age groups	Date	Weight of sample	Number of larvæ	Average weight of 1 larva	Extract used <sup>a</sup>	Equivalent number of larva	CuSO <sub>4</sub> solution	Equivalent dextrose	Dextrose per larva	Sugar per larva
2.....	Gram 0.004745	Gram Up to 0.014685.	1922 7-18	Gram 0.6233	50	0.01247	Cc. 50	50	Cc. 5	Gram No reaction.	Gram 0	P. ct. 0
3.....	.024626	0.014685 to 0.059308.	7-25	.4967	25	.01987	50	25	5	No reaction.	0	0
			7-25	1.1072	25	<sup>b</sup> .04429	44	22	5	0.01020	0.000463	1.13
			8-2	<sup>b</sup> 1.0979	25	<sup>b</sup> .04392	23.5	23.5	5	.01020	.000434	.98
			5-9	<sup>c</sup> 2.2906	50	.04581	90	45	10	.02040	.000453	.94
			8-11	1.1706	25	.04682	42	21	5	.01030	.000490	1.04
			7-27	1.4009	25	.05604	20.25	10.125	5	.01020	.001007	1.79
	Average	-----				.043222					.000475	.98
4.....	0.093990	0.059308 to 0.120369.	8-2	1.6749	25	.06700	21	10.5	5	.01020	.00097	1.44
			8-11	<sup>d</sup> 1.6817	25	.06727	11	9.16	5	.01030	.00112	1.66
			8-11	1.9372	25	.07749	12.75	6.375	5	.01030	.00161	2.07
			8-11	1.9916	25	.07966	18.1	9.05	5	.01030	.00113	1.41
			7-31	2.3044	25	<sup>a</sup> .09218	6.8	3.4	5	.01020	.00300	3.25
			8-2	2.3566	25	<sup>e</sup> .09426	8.6	4.3	5	.01020	.00237	2.51
			8-30	2.3733	25	.09493	6.58	3.29	5	.01030	.00313	3.29
			7-25	2.4901	25	.09960	5.8	2.9	5	.01020	.00351	3.52
			8-2	2.6748	25	.10699	8.0	4.0	5	.01020	.00255	2.37
			8-17	2.6843	25	.10737	4.75	2.38	5	.01030	.00431	4.01
			8-18	2.7781	25	.11112	4.35	2.175	5	.01030	.00473	4.25
			8-2	2.7919	25	.11168	7.0	3.5	5	.01020	.00291	2.61
			8-18	2.8205	25	.11282	4.35	2.175	5	.01030	.00473	4.19
			6-1	2.8332	25	.11333	12.7	6.35	8.9	.018156	.00286	2.52
			8-11	2.8972	25	.11589	6.8	3.4	5	.01030	.00303	2.61
			8-4	2.9274	25	.11710	7.1	3.55	5	.01020	.00287	2.45
			8-2	2.9505	25	.11802	7.1	3.55	5	.01020	.00287	2.43
			8-31	2.9749	25	.11900	5.2	2.6	5	.01030	.00396	3.32
			8-2	2.9908	25	.11963	6.0	3.0	5	.01020	.00340	2.84
			8-17	3.0038	25	.12015	4.5	2.25	5	.01030	.00457	3.80
			8-4	3.0105	25	.12042	6.5	3.25	5	.01020	.00314	2.61
	Average	-----				.10314					.00299	2.82
5.....	0.146748	0.120369 to 0.150876.	6-1	<sup>c</sup> 3.0961	25	.12384	27.0	6.75	10	.02040	.00317	2.57
			8-18	3.1148	25	.12459	4.2	2.1	5	.01030	.00490	3.93
			8-18	3.1953	25	.12781	4.2	2.1	5	.01030	.00490	3.83
			8-11	3.2141	25	.12856	6.4	3.2	5	.01030	.00322	2.51
			7-26	3.3153	25	.13261	5.6	2.8	5	.01020	.00364	2.75
			7-31	<sup>e</sup> 3.3278	25	.13311	5.0	2.5	5	.01020	.00408	3.06
			8-11	3.3479	25	.13392	6.5	3.25	5	.01030	.00317	2.36
			8-10	3.3602	25	.13441	6.15	3.075	5	.01030	.00334	2.45
			7-31	3.3689	25	.13476	4.1	2.05	5	.01020	.00497	3.69
			8-11	3.3706	25	.13482	6.1	3.05	5	.01030	.00337	2.49
			7-27	3.3721	25	.13488	4.5	2.25	5	.01020	.00453	3.35
			7-25	3.4029	25	.13612	3.4	1.7	5	.01020	.00600	4.41
			7-25	3.4620	25	.13848	5.25	2.625	5	.01020	.00388	2.80
			8-17	3.4644	25	.13858	3.5	1.75	5	.01030	.00588	4.23
			8-31	3.4775	25	.13910	4.6	2.3	5	.01030	.00448	3.22
			7-25	<sup>f</sup> 3.6156	25	.14462	7.92	3.96	6.07	.01238	.00312	2.15
			8-17	3.6394	25	.14558	3.6	1.8	5	.01030	.00572	3.92
			8-17	<sup>f</sup> 3.6971	25	.14788	4.2	2.1	5	.01030	.00490	3.31
			8-18	<sup>f</sup> 3.7164	25	.14866	5.2	2.6	5	.01030	.00396	2.66
	Average	-----				.13591					.00428	3.14

<sup>a</sup> Unless otherwise stated, total cc. of extract equals 50. <sup>d</sup> Total extract, 30 cc. only.  
<sup>b</sup> Total extract, 25 cc. only. <sup>e</sup> Just sealed, early.  
<sup>c</sup> Total extract, 100 cc. <sup>f</sup> Just sealed, still coiled.

TABLE V.—Unassimilated sugar in intestinal content of larvæ at different ages—Continued

Larvæ of knownage, Sturtevant (35)			Larvæ analyzed for presence of unassimilated sugar (weights in grams)									
Age in days	Average weight	Limits by weight for age groups	Date	Weight of sample	Number of larvæ	Average weight of 1 larva	Ex-tract used	Equiv-alent number of larva	CuSO <sub>4</sub> solution	Equiv-alent dex-trose	Dex-trose per larva	Sugar per larva
6-----	Gram 0.155005	Gram 0.150876 to maximum and down to 0.148326.	1922	Grams		Gram	cc.		cc.	Gram	Gram	P.ct.
			7-18	3.8012	25	0.15205	18.05	4.51	9.6	0.19584	0.00434	2.85
			8-31	3.8038	25	.15215	5.4	2.7	5	.01030	.00381	2.50
			8-11	3.8925	25	.15570	4.35	2.175	5	.01030	.00473	3.03
			8-31	3.9783	25	.15913	6.0	3.0	5	.01030	.00343	2.15
			8-31	4.1249	25	.16500	7.0	3.5	5	.01030	.00294	1.78
			7-27	3.7706	25	.15082	6.65	3.33	5	.01020	.00306	2.03
			Average	-----	-----	.15581	-----	-----	-----	-----	.00372	2.39
	0.141648	0.148326 to 0.139406.	8-18	3.6980	25	.14792	7.3	3.65	5	.01030	.00282	1.91
			8-10	3.5600	25	.14280	8.9	4.45	5	.01030	.00231	1.56
8-10			3.5835	25	.14334	11.5	5.75	5	.01030	.00179	1.25	
8-4			3.5572	25	.14229	7.4	3.7	5	.01030	.00275	1.93	
8-18			3.4871	25	.13948	45.0	22.5	5	.01030	.00050	.36	
Average			-----	-----	.14397	-----	-----	-----	-----	.00203	1.40	
0.137165	0.139406 to 0.135158.	7-26	3.4358	25	.13743	50.0	25	5	No re-action.	0	0	
		7-18	3.4453	25	.13781	50.0	25	5	No re-action.	0	0	
		Average	-----	-----	.13762	-----	-----	-----	-----	0	0	
0.133152	-----	8-4	3.3232	25	.13293	50.0	25	5	No re-action.	0	0	

c Total extract, 100 cc.  
• All sealed, coiled or with backs out. Feeding ended and spinning of cocoons started.  
^ Cocoon partially spun, still some color in the intestine.  
^ Cocoon not quite finished, still moving somewhat, no color in intestine.  
/ Quiescent prepupæ, intestines colorless, empty, histolysis started.  
^ First indication of change in external form.

OBSERVATIONS

Over 60 samples of 25 larvæ each of various ages, containing over 1,600 individual larvæ, were analyzed for the presence of reducing sugars. The largest number of analyses were made on larvæ from 3½ to 5½ days of age during the active honey and pollen feeding period. At least five analyses were made of each of the other age periods which might show the presence of sugar. To obtain averages with a small probable error, the analyses are grouped by age periods of 24 hours each, as described earlier (Table V, fig. 13). All larvæ in the two-day group, as well as one sample of larvæ nearly as heavy as the three-day average larva, showed no reducing sugar. Larvæ in the three-day group, averaging 0.043222 gm. in weight, gave 0.000475 gm. of reducing sugar per larva, or 0.98 per cent concentration. Larvæ in the four-day group, averaging 0.10314 gm. in weight, gave 0.00299 gm. of reducing sugar per larva, or 2.82 per cent concentration. Larvæ in the five-day group, comprising those just prior to sealing, with a few just sealed, averaging 0.13591 gm. in weight, gave 0.00428 gm. of reducing sugar per larva, or 3.14 per cent concentration. In the five-day group there were two samples which gave a concentration of over 4 per cent, the maximum being 4.41 per cent. The six-day group, comprised entirely of larvæ that had been sealed, had finished feeding and had started spinning, averaging 0.15581 gm. in weight, gave 0.00372 gm. of reducing sugar per larva, or 2.39 per cent concentration. This group contains larvæ of maximum size (fig. 14). From

this point on the gross weight decreases as preparation for metamorphosis begins. The seven-day group, comprising larvæ which are still moving about in spinning, and most of which show only a slight remaining color in the intestines, indicating

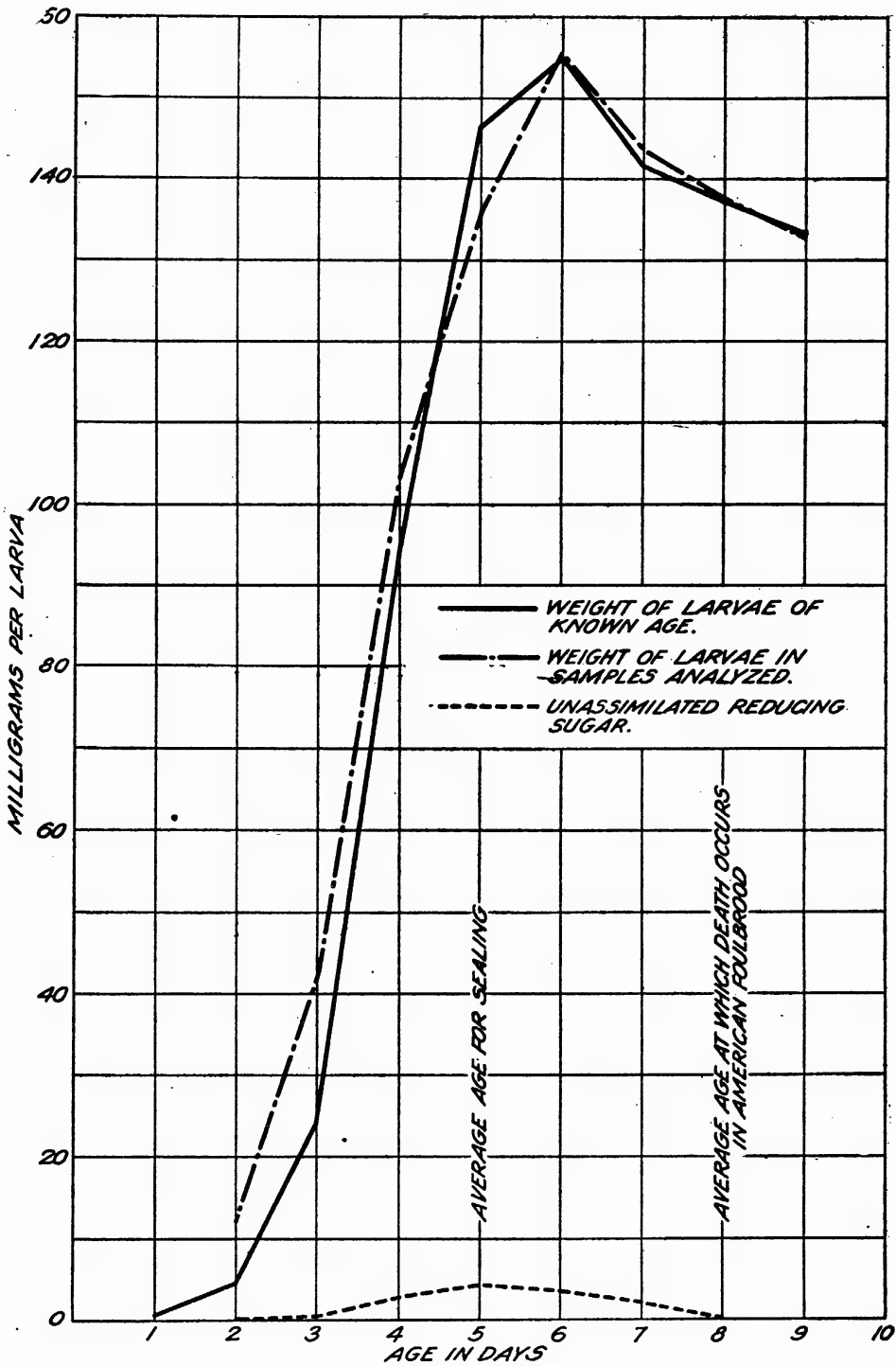


FIG. 13.—Unassimilated sugar in larvæ at different ages (Table V)

that the connection between ventriculus and end gut is made, averaging 0.14397 gm. in weight, gave 0.00203 gm. of reducing sugar per larva, or 1.40 per cent concentration. One sample in this group gave as low as 0.36 per cent. Larvæ

of the eight-day group, averaging 0.13762 gm. in weight, showed a total absence of reducing sugar. These larvæ represent the two-day quiescent prepupal stage (fig. 3 and 4). They have stretched out motionless in the cell, the intestines are entirely empty and colorless, and the histolysis of the tissues preliminary to metamorphosis has begun.

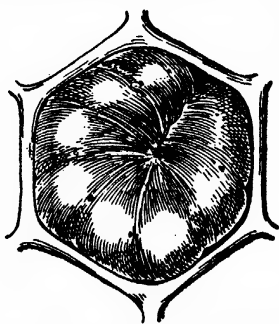


FIG. 14.—Healthy larva at age of maximum size, just after sealing and before the start of the cocoon-spinning period (White (56))

From these observations it is seen that there is an amount of reducing sugar in the entire actively feeding larva which would seriously interfere with the germination and growth of *Bacillus larvae*, provided the entire bee larva were to serve as the medium for its growth. Since this reducing sugar does not exist equally distributed throughout the bee larva, and since at this stage the organisms are found almost solely in the intestinal tract, it is certain that the reducing sugar concentration of the intestine is sufficient to prevent the germination of *Bacillus larvae*, so that death from American foulbrood is

delayed until after the larva has been sealed in the cell and has become quiescent. This will be discussed more in detail later.

#### SUPPLEMENTARY STUDIES ON THE BIOCHEMICAL REACTIONS OF *BACILLUS* LARVAE

Up to the present time few facts have been determined concerning the biochemical reactions of *Bacillus larvae*, mainly because of a lack of suitable culture media. White states (55), "Carbohydrate liquid media as ordinarily prepared are not suitable for the growth of *Bacillus larvae*. In some of these after a considerable period a slight growth may appear at the bottom of the tubes. A little brood-filtrate or egg-suspension added to the media improves it. No visible gas is formed, but in some instances slight acidity is produced. No growth takes place in plain or in brood-filtrate gelatin at temperatures at which it remains congealed." Maassen states (28), "The bacillus also grows on nutrient gelatin. Upon a nutrient gelatin medium which had been made from the previously mentioned nutrient liquids, and an almost completely neutralized gelatin (a so-called emulsion of gelatin), there resulted growth although very slowly, from which a quite gradual liquefaction of the gelatin resulted. Liquefaction did not occur in the presence of grape sugar (dextrose). Through the addition of 1 per cent grape sugar the growth-producing ability of the gelatin as well as of other nutrient media was noticeably improved. On the most favorable media no special chemical properties were shown, with the exception of the ability to peptonize. The destruction of the albuminous bodies occurred very slowly and with little characteristic appearance. Only in worn-out cultures could any odor resembling foul glue be detected after a time." There are, however, certain characteristic manifestations in American foulbrood resulting from the growth and metabolism of *Bacillus larvae*, aside from the gross symptoms and appearances, which only a more complete knowledge of the biochemical activity of the organism can explain.

From the previous cultural experiments (Table I) it may be seen that apparently *Bacillus larvae* can utilize in its metabolism a certain amount of reducing sugar (dextrose), although this sugar is not necessary to the development of the organism. In the larva which is attacked by American foulbrood there may be two sources of sugar, that present unassimilated in the intestine and that hydrolyzed from the stored glycogen. Hydrolysis of glycogen may occur in connection with histolysis of the tissues preparatory to metamorphosis through enzym

action, or *Bacillus larvae* itself may have the ability to produce enzymes which hydrolyze the glycogen, or it may be a combination of both. Through the utilization of this reducing sugar one would expect that there at least would be a considerable production of acid, but, as stated earlier, the hydrogen-ion concentration of dead ropy material is never found to vary much from  $P_H=6.6$  to 6.8. Since the data available concerning the biochemical reactions of *Bacillus larvae* offer no explanation of this hydrogen-ion concentration, a series of experiments was devised, the results of which add materially to the knowledge concerning the biochemical reactions and relationships of *Bacillus larvae*. In certain cases where, because of the limitations on growth, cultural growth has failed, it was found possible to obtain the desired information by examination of the diseased larval remains.

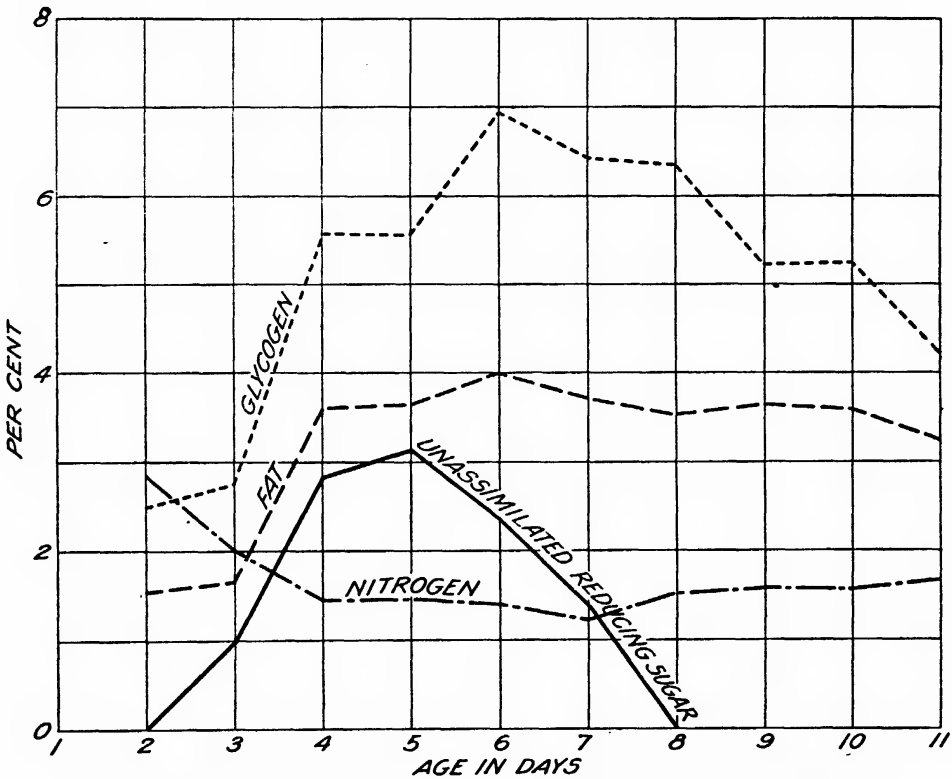


FIG. 15.—Per cent composition of worker larvæ at different ages (Tables IV and V)

UTILIZATION OF GLYCOGEN

According to Straus (Table IV, fig. 15) the greatest percentage of stored glycogen occurs just after sealing, when feeding has ceased. If an emulsion of the tissues of a larva of this age, or slightly older, at the age when prepupæ usually die of American foulbrood, is tested for the presence of glycogen with iodine solution,<sup>4</sup> the resulting deep reddish brown color shows that there are large amounts of glycogen present. If a prepupa which has just died from disease, slimy in consistency, light brown in color, and which in the microscopic picture still shows the presence of vegetative rods, is tested with iodine solution, it will

<sup>4</sup> Glycogen treated with iodine solution gives a color varying from brown to wine red, which disappears upon heating to 60° C., but returns again upon cooling. Soluble plant starch with iodine solution gives the following reactions: Amylodextrin, first dextrin of conversion, dark blue; erythrodextrin, second dextrin of conversion, red; intermediate steps give various shades of purple or lavender.

be found that most of the glycogen has disappeared, although the iodine solution gives a light yellowish brown color. The presence of a trace of reducing sugar also occasionally can be demonstrated with Benedict's solution in diseased material of this type where vegetative organisms are still actively present. In material which has decomposed completely, has reached the dark brown ropy stage (fig. 9), and contains only spores of *Bacillus larvae*, glycogen is found to be completely absent, nor can any reducing sugar be demonstrated, the sugars having been completely destroyed.

This type of material stained with Sudan III or osmic acid (32, p. 78) shows fat globules in practically the same condition and amount as in healthy larvae, so that fat is apparently not acted upon by *Bacillus larvae* even after drying down to the scale stage.

Glycogen of the fat body of the healthy larva is hydrolyzed to dextrose to be used in metamorphosis, by the action of enzymes during the histolytic processes subsequent to sealing and prior to metamorphosis. This enzyme action is demonstrated by the following experiments:

#### EXPERIMENTAL PROCEDURE

Several series of 50 healthy prepupæ each that had reached the period of quiescence were macerated in 25 cubic centimeters of 50 per cent alcohol and incubated at 37° C. for from 3 to 24 hours. The extract was then filtered and diluted with an equal amount of water. A series of test tubes were prepared, using for each tube 5 cubic centimeters of this extract and 5 cubic centimeters of 0.4 per cent glycogen in water, and also another series using 5 cubic centimeters each of a 0.1 per cent soluble starch. Both glycogen and starch were used, since it has been shown by Bradley and Kellersberger (8), as well as by experiments by the writer using commercial Taka-diastase, that diastase acts similarly on both glycogen and starch. These tubes were incubated for various periods and then tested with iodine solution for the presence of glycogen and starch (Table VI). Hydrolysis of both glycogen and starch seems to be complete after incubation for about five hours, and positively complete after incubation overnight, demonstrating the presence of diastase in the prepupæ.

In another experiment 50 prepupæ were macerated in 50 cc. of water and incubated at 37° C. for 24 hours. Then sufficient 95 per cent alcohol was added to precipitate any glycogen present, and the solution was filtered and tested with both the qualitative and the quantitative Benedict's solutions. In both cases definite traces of reducing sugar could be demonstrated, none having been present in the original solution before incubation, again demonstrating enzyme activity of the larval tissues. This may have been due to action by bacterial contamination, but if such had been the case the sugar would probably have been fermented and could not have been demonstrated.

In a similar manner extracts with 50 per cent alcohol were made of ropy diseased material, enzyme activity being demonstrated in the same manner as above. This, however, does not indicate whether the organism causing the disease has any diastatic power or whether the reaction was due to enzymes remaining in the decomposed tissues. Further extracts were made with 25 per cent and 50 per cent alcohol of several 48-hour vegetative cultures of *Bacillus larvae* grown on egg-yolk suspension medium. These extracts showed definite enzyme activity with glycogen after a few hours' incubation, and more positive activity after incubation overnight (Table VI), while with starch marked hydrolysis was shown after only a few hours' incubation.



TABLE VI.—Test for diastatic action with alcoholic extract<sup>a</sup>

Test material	Control tubes		Color with iodine after incubation of—									
			0 hour		½ hour		2½ hours		5½ hours		18 hours	
	Glycogen	Starch	Glycogen	Starch	Glycogen	Starch	Glycogen	Starch	Glycogen	Starch	Glycogen	Starch
Extract of healthy prepupæ.....	+++++	+++++ (blue)	+++	++	++	+	+	±				
Extract of decomposed rosy remains.....	+++++	+++++			++	+	+	±				
Extract of vegetative cultures.....	+++++	+++++			+++	++	++	+				

<sup>a</sup> The following symbols are used:  
 +++++ Deep color, brown or blue.  
 ++++ Slightly lighter brown than check or wine color.  
 ++ Light coffee brown or lavender.  
 + Trace faint brown or trace faint lavender.  
 — No color or only iodine color, showing complete diastatic action.

To further determine the production of diastase by *Bacillus larvae*, a series of Petri dishes were poured, using yeast-extract egg-yolk suspension agar, to which had been added respectively 0.25 per cent and 1 per cent of glycogen and 0.25 per cent and 1 per cent of starch, this being an adaptation from methods described by Vedder (48) and by Allen (1). After solidification of the media in the Petri dishes, smears were made upon the surface of the agar from 48-hour cultures of various previously isolated strains of *Bacillus larvae*. After several days the plates were examined, first by holding up to the light and then later by flooding with iodine solution, and comparing with control plates containing no starch or glycogen. In nearly all the plates good growth had occurred, causing clear areas to be produced in the cloudy culture medium extending slightly beyond the edge of the area of growth. When flooded with iodine the halo around the culture growth, although not wide, was more prominently differentiated from the surrounding medium, showing in both glycogen and starch plates. These results, in conjunction with those of the extraction experiments, demonstrate that weak diastatic action is produced by *Bacillus larvae*.

### ACID PRODUCTION

It has been shown that there is still an appreciable amount of sugar (reducing sugars in the food remaining in the intestines and dextrose available from glycogen) present in the larva after sealing and in the prepupa at the age when American foulbrood attacks, available for fermentation (Tables IV and V). In the various cultural investigations both by others and by the present writer, there is no evidence of carbon dioxide production. It would be expected, however, that at least some acid would be produced from the bacterial fermentation of these sugars, which is known to be present. To determine this more definitely than heretofore, a culture medium was devised for the qualitative determination of acid production, which gave good vigorous growth of *Bacillus larvae*.

The method used is an adaptation of the method of using agar slants for detecting acid formation, instead of liquid medium, described by Conn and Hucker (18), in which the change in reaction can readily be seen. The regulation yeast-extract egg-yolk suspension agar was prepared for this purpose by adding to the yeast extract base before sterilization an indicator in the proper amount both to the plain medium and also to a portion to which was added 1 per cent of dextrose.

Brom thymol blue was first used, as it covers the range of the supposed optimum reaction for *Bacillus larvæ* as described earlier. Baker (4) also has shown that brom thymol blue, used in about a 0.0024 per cent concentration in culture media, gives the most desirable color for comparison, without inhibiting acid fermentation. This concentration was obtained by using 12 cc. of a 0.2 per cent alcoholic solution of the indicator per liter. After marked acid production in the dextrose tubes was demonstrated with brom thymol blue, brom cresol purple was used as suggested by Conn and Hucker (18) in a 0.001 per cent concentration as a check on the end point. This concentration was obtained by using 8 cc. of a 0.2 per cent alcoholic solution of the indicator per liter. The yeast-extract base, both with and without dextrose, was adjusted so that after the addition of the egg-yolk suspension the final medium would have a primary reaction of approximately  $P_H=7.2$ , a definite blue grass green in the case of brom thymol blue and a marked purplish tinge with brom cresol purple, except in one series, where the primary reaction of the plain medium was  $P_H=7.6$ . These tubes after being slanted were inoculated as usual, both with vegetative cultures and with diseased material containing spores. The change in reaction was noted after different lengths of incubation, and the final reaction was determined by comparison with standard buffer tubes used in combination with tubes of plain egg-yolk suspension media slanted in the same manner. The approximate increase in hydrogen-ion concentration was determined by this comparison (Table VII).

TABLE VII.—Acid production by *Bacillus larvæ*

Culture No.	Brom thymol blue indicator				Brom cresol purple indicator			
	Plain medium		1 per cent dextrose		Plain medium		1 per cent dextrose	
	Control	Inoculated	Control	Inoculated	Control	Inoculated	Control	Inoculated
	$P_H$	$P_H$	$P_H$	$P_H$	$P_H$	$P_H$	$P_H$	$P_H$
9693-1.....	7.6	6.8	7.2	6.0	(b)	(c)	(d)	5.8
9834-1.....	7.2	6.6	7.2	6.0				
9834-2.....	7.6	7.4	7.2	6.2	(b)	(c)	(d)	6.0
9853.....	6.8-7.0	±6.6	6.6	6.0				
9857.....	7.2	6.6-6.8	7.2	6.0-6.2				
9867.....	7.6	7.4	7.2	6.4	(b)	(c)	(d)	6.2
9869.....	7.6	7.4	7.2	6.0	(b)	(c)	(d)	5.8
9874.....	7.6	7.0-7.2	7.2	6.0	(b)	(c)	(d)	5.8

<sup>a</sup> Doubtful growth.  
<sup>b</sup> Beyond end point, no growth.  
<sup>c</sup> No change in color, good growth.

<sup>d</sup> Beyond end point, good growth.  
<sup>e</sup> No change in color, no growth.

OBSERVATIONS

Several interesting facts were observed from these experiments. Addition of buffer salts to the media delayed the approach to the final hydrogen-ion concentration reaction somewhat, but eventually practically the same end point was reached. Also, in one series of media in which the plain medium was adjusted to about  $P_H=7.6$ , little if any growth occurred in these tubes except with two strains of *Bacillus larvæ*, indicating that the alkaline limit for growth is about at this point. In cases where the initial reaction of the plain medium was  $P_H=7.2$ , the final reaction averaged  $P_H=6.6$  to  $P_H=6.8$  (Table VII). In the case of the medium to which 1 per cent dextrose had been added, the final reaction averaged about  $P_H=6.0$  for brom thymol blue and from  $P_H=5.8$  to  $P_H=6.0$  for brom cresol purple (Table VII). While, therefore, only a slight change in reaction occurred in media without sugar, a marked production of

acid was indicated in the tubes to which 1 per cent dextrose had been added. The maximum production of acid, however, required approximately 48 hours or more, the fermentation of the sugar apparently being relatively slow. As has been stated, however, the reaction of diseased material in various stages of decomposition and drying down is never found to reach a hydrogen-ion concentration of more than  $P_H = 6.6$ , and usually averages  $P_H = 6.8$ .

### PROTEIN DECOMPOSITION

It is known that certain organisms have the ability to break down protein material under proper conditions, with the production of amino acids and alkaline decomposition substances, which latter tend to neutralize any acid produced from fermentation of sugar. If it can be shown that *Bacillus larvae* has this ability, it will explain the fact that the remains of larvae dead from American foulbrood do not show a greater acid reaction resulting from the fermentation of the sugar of the intestinal contents. A series of experiments was devised to demonstrate whether such is the case with *Bacillus larvae*.

The prepupa at the age attacked by American foulbrood contains nitrogenous substances as shown by the Kjeldahl nitrogen determination equivalent to 1.45 per cent nitrogen (43). The source of this nitrogen is mainly albuminous material, one of the constituents of the larval fat body. Its exact composition has not been determined, but without doubt it is complex in nature. There are certain color reaction tests by means of which the constitution of this nitrogenous material may be indicated.

A delicate test for the presence of coagulable protein is that of Heller (32, p. 1067). A suspension of healthy prepupæ in water, treated by pouring about 4 cc. of concentrated nitric acid down the side of the inclined test tube, causes a white ring to form at the junction of the two liquids. Decomposed ropy material tested in this way gives no indication of such a ring, indicating that the complex protein has disappeared.

One of the most characteristic reactions for complex protein is the biuret test (32, p. 915). If some healthy prepupæ are suspended in a few cubic centimeters of 10 per cent sodium hydroxid and are treated with a few drops of a 0.5 per cent copper sulphate solution, a distinct pinkish-violet color is formed, again indicating the presence of complex protein material. Decomposed ropy material tested in this way gives no indication of this color, again indicating the complete disappearance of the complex protein.

There is also the xantho-proteic reaction (32 p. 916), which is given both by solid and by dissolved protein, and indicates the presence of the amino-acids, tryptophan, tyrosin, or phenylalanin in the protein molecule, or in solution. Tryptophan gives the reaction most intensely. Both healthy prepupæ and ropy material, boiled with concentrated nitric acid, produce a lemon-yellow color which on cooling and neutralizing with sodium hydroxid changes to an orange, denoting a positive reaction.

An even more delicate reaction for protein is that with Millon's solution (32, p. 916). A few cubic centimeters of a suspension of healthy prepupæ, treated with a few drops of Millon's reagent and boiled, cause a brick-red precipitate to form, leaving the liquid practically clear. A solution of decomposed ropy material, treated in the same way with Millon's reagent and boiled, causes a somewhat similar reddish precipitate, but the solution is also distinctly colored similarly, indicating that the protein has been changed in some way, part at least being soluble in water. Tyrosin is the only amino acid in protein that gives this reaction.

Since tryptophan is probably one of the principal constituents of the protein molecule in the healthy prepupa as well as in solution in diseased material, certain tests were made to determine its presence, because this amino-acid is easily utilizable by bacteria and gives decomposition products indicating the nature of bacterial action. The following tests are specific tryptophan reactions:

*Adamkiewicz reaction* (32, p. 917).—A suspension of healthy prepupæ or of diseased material in glacial acetic acid, treated by pouring concentrated sulphuric acid down the side of the inclined tube, causes a violet ring to form at the junction of the two liquids, indicating the presence of tryptophan, either as part of the complex molecule or in solution.

*Rhodes reaction* (41).—To a suspension of healthy prepupæ or of diseased material in water, a few drops of a weak solution of dimethylaminobenzaldehyde is mixed and concentrated sulphuric acid poured down the side of the inclined tube. This produces a violet ring at the junction of the two liquids which, if shaken, produces a reddish violet coloration in the mixture.

#### PROTEIN DECOMPOSITION PRODUCTS

It is therefore evident that the composition of the nitrogenous material in the healthy prepupæ is more or less complex but that certain amino-acids are available for bacterial metabolism, or are produced as a result of bacterial action.

In the decomposition of nitrogenous material, however, certain bacteria have the power of breaking down these amino-acids, such as tryptophan, to more simple compounds, some of them alkaline in nature, and often more or less foul smelling, or even to break them up into ammonia, the final product of nitrogenous decomposition. Indol is one of the products of such action of bacteria on material containing tryptophan. Its determination is largely used in the characterization of various organisms (36). Two indol tests were used, Ehrlich's aldehyde test (19) and the vanillin test (19), using for both suspensions of diseased material as well as cultures. Test of suspensions of diseased material gave positive results for the presence of indol, both with the Ehrlich method and even more definitely with vanillin. For testing in pure culture a broth consisting of 2 per cent peptone, 10 per cent yeast extract, and a few cubic centimeters of egg-yolk suspension was inoculated, incubating at 37° C. for about one week. Growth took place in this broth sufficiently to give a slight positive pink color with the Ehrlich aldehyde test, increasing on standing, and a much more positive result with the vanillin test.

#### AMMONIA PRODUCTION

Test of a suspension of diseased material as well as some of the above culture broth with Nessler's reagent (32, p. 1084) for presence of ammonia gave indications, from the resulting slight production of characteristic yellowish color, that the decomposition had passed even to the ammonia stage. A more delicate qualitative test was devised, using the modification of the microchemical method of Folin and McCallum (32, p. 1093) for the determination of urinary ammonia as described by Steel (42). To 25 cc. of a suspension of diseased material, or to broth culture similar to the above, 1 gram of sodium hydroxid and 15 grams of sodium chlorid are added and ammonia-free air bubbled through into 20 cc. of an approximately N/20 sulphuric acid, to which 10 drops of the indicator thymol blue are added. This showed the sulphuric acid solution to have a primary hydrogen-ion concentration of about  $P_H=2$ . After bubbling air through for an hour or more, in the case of the decomposed rosy material, sufficient ammonia had been carried over to neutralize part of the acid and change the hydrogen-ion concentration reaction from  $P_H=2$  to  $P_H=2.8$  or 3. Also one culture out of

three showed a change from  $P_H=2$  to  $P_H=2.8$ . Therefore apparently *Bacillus larvae* has the ability of producing at least small amounts of ammonia. It seems probable that the rather pungent volatile glue-like odor often associated with American foulbrood receives some of its characteristics from this ammonia as well as from certain of the protein digestion products.

#### GELATINE LIQUEFACTION

The ability of putrefactive bacteria to liquefy gelatin is difficult to demonstrate with *Bacillus larvae* because of the cultural limitations. Maassen states (28) that slow liquefaction takes place, while White (55) was unable to demonstrate any growth in gelatin. The writer inoculated a number of tubes of plain gelatin with several strains of *Bacillus larvae*, all of which showed slight growth, and one or two showed a slight softening of the gelatin about the culture growth. Tubes of gelatin to which some egg-yolk suspension was added showed this softening more markedly, but in no case was there sufficient liquefaction to enable one to say that it was positive. Decomposed rosy material inoculated into plain gelatin, on the other hand, gives a marked liquefaction in a short time. This, however, probably is due not to enzymes produced by *Bacillus larvae* so much as to enzymes from the body tissues functioning in the histolysis previous to metamorphosis.

This series of experiments, however, demonstrates that sufficient alkaline decomposition products are formed by the action of *Bacillus larvae* in the prepupa to neutralize most of the acid formed by the fermentation of the sugar in the intestinal contents and the dextrose resulting from the hydrolysis of the stored glycogen.

#### DISCUSSION

##### PER CENT CONCENTRATION OF SUGAR

In the data presented it may be seen that there is not an exact correlation between the percentage of dextrose which inhibits the germination or prevents the growth of *Bacillus larvae*, and the percentage of unassimilated sugar in the larva as expressed. The reason for this is that the percentage of unassimilated sugar is calculated in relation to the entire weight of the larva, like the figures of Straus (43) on the percentage composition of the larva (fig. 15). The percentage of dextrose in the culture media gives the actual effective concentration of the sugar in the medium by weight. Since the unassimilated sugar is contained almost entirely in the intestine from which it is absorbed, the true concentration of sugar in the intestine should be determined in relation to the weight of the intestinal content. Furthermore, as suggested by Maassen (28), growth of *Bacillus larvae* occurs only inside the intestine until after the histolysis has begun, making possible the invasion of the body tissues by the organisms. It is therefore in the intestinal contents during the last part of the feeding period that the presence of sugar is primarily effective in inhibiting the growth of the organisms. The actual concentration of sugar in the intestine is, however, difficult to determine accurately, since the actual weight of food consumed by the larva for each 24 hours of the feeding period is unknown. Furthermore, the weight of the intestinal content is difficult to determine, because of the difficulty of dissecting the intestine free from the surrounding body tissues or of removing the contents intact.

Several attempts were made, however, to remove intestines with as little adhering tissue as possible from larvae of different sizes during the last two days prior to sealing, in order to obtain an approximately accurate figure for the relation between the weight of the intestine and the weight of the larva. This in the several larvae dissected was found to be almost always about 1 to 5. Using this factor,

the percentage concentration in the intestine, at least during the progressive feeding period, should be approximately five times as great as the value calculated (in Table V) on the basis of the entire larval weight. The calculated percentages for the third and fourth days are now 4.90 and 14.10, respectively, and on the fifth day, just before sealing, the sugar concentration in the intestine should approximate 15.70 per cent (fig. 16). There is, of course, the factor of dilution,

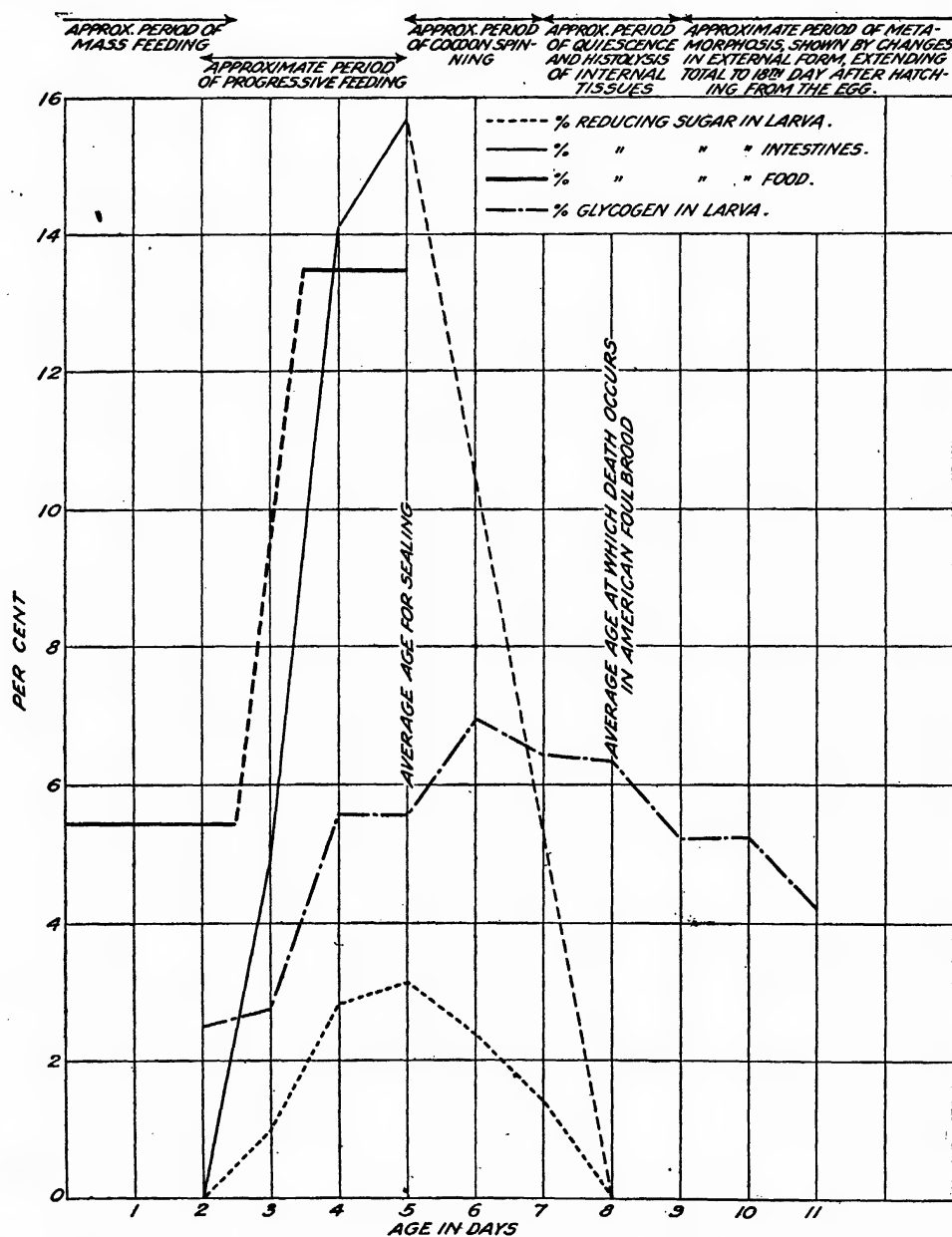


FIG. 16.—Correlation of time of death from American foulbrood to per cent concentration of reducing sugar in entire larva and to calculated per cent concentration in the intestine. Per cent sugar in the food and per cent glycogen in the larva are shown for comparison

particularly toward the end of the feeding period, caused by the accumulation of undigested pollen shells, which may lower this figure somewhat.

Still another approximate check may be calculated from the molecular weights of dextrose, glycogen, and fat, and from the percentage composition of the constituents of the larva (Tables IV and V), in order to obtain the percentage of sugar which was present in the intestine at any one time previous to assimilation neces-

sary for the formation of the stored glycogen and fat, on the basis of the relation of their carbon atoms.

	For- mula	Molec- ular weight	Equivalent sugar per cent	
Dextrose (unassimilated).....	$C_6H_{12}O_6$	= 180		<sup>5</sup> 3. 14
Glycogen.....	$C_5H_{10}O_5$	= 162	$\frac{180-162}{180} \times 25.57 + 5.57 =$	6. 13
Fat (oleic acid).....	$C_{18}H_{34}O_2$	= 282	$\frac{3 \times 180 - 282}{3 \times 180} \times 63.64 + 3.64 =$	5. 38
Total.....				14. 65

Since more sugar is used for energy in the production of a molecule of glycogen or of a molecule of fat (32, p. 77) than is indicated by the actual relation of the carbon atoms, this figure should be somewhat higher, thereby more nearly corresponding with the figure calculated from the weight ratio between intestine and larva.

The average concentration of sugar in the food of a larva of the age during which inhibition of bacterial growth takes place is 13.48 per cent (40) (Table III, fig. 11). The percentage composition of the larva at the different age periods (Table IV, fig. 15) in relation to food composition indicates, however, that the food probably is not assimilated as rapidly as it is ingested by the larva. If this is the case, an increase in the unassimilated sugar in the intestine would occur, as is indicated by the results obtained and the calculated percentage figures. The percentage of glycogen and fat increases slightly by the third day, as a result of the change in the composition of the food and the resulting increase in nursing (26). This is accompanied by the appearance of unassimilated sugar in the larva (Table V, fig. 15). There is then a marked increase by the fourth day, when apparently the limit for assimilation is reached, as shown by the constant percentage of glycogen in the larva as a whole between the fourth and fifth days in spite of the increase in weight, until after feeding ceases, when there is another increase in storage during the next 24 hours (sixth day). The latter is the result of the consumption of the food remaining in the cell after capping. The amount of unassimilated sugar increases continually, however, until feeding ceases, soon after the larva is sealed in the cell. This fact probably accounts for the slight difference between the percentage of sugar in the food and the calculated percentage in the intestine, but the correspondence is so striking as to substantiate the assumption.

Even though these calculations are only approximately accurate, it is known that some such condition must exist, since observations which have been made on the nursing habits of the honeybee (26), considered in relation to the figures for unassimilated sugar and food composition, give adequate foundation to the conclusion that there is considerably more than enough sugar in the intestine at the time infection occurs, or soon after, to inhibit the growth of the organism causing American foulbrood.

#### THE ACTIVE FEEDING STAGE IN THE LIFE HISTORY OF THE LARVA

The feeding stage of the honeybee larva has been divided into two parts, described by Lineburg (26) as the mass feeding period and the progressive feeding period. These two periods are characterized by a difference in the manner of feeding and in the amount of time spent by the nurse bees in the process, as well as by the change in chemical composition and character of the food (40) (Table III), and by the chemical composition of the larvæ themselves (43) (Table IV). It has been determined, however, that this change in composition of food occurs

<sup>5</sup> From Table V.

<sup>6</sup> From Table IV, Straus.

much earlier than stated by Von Planta (35, 26). The young larvæ receive a food rich in nitrogenous material and relatively low in sugar and in which pollen grains are absent during about the first two and a half days of larval life. A large part of this, which is several times in excess of the weight of the larva during the first 24 hours or more (35) (Table VIII), seems to be placed in the cell with the newly hatched larva at one time soon after hatching, which justifies the assumption of mass feeding. During this period, assimilation must be very rapid, because the greatest relative growth occurs during the first two to three days (Table VIII) and also because no unassimilated sugar can be demonstrated in the larva during this period, even though the food contains about 5 per cent reducing sugar (Table V). The high nitrogenous content of the food apparently serves for rapid cell building, while the sugar is largely consumed in producing energy for this rapid growth; little storage of glycogen or fat occurs during this period. The nature and composition, as well as the biochemical reactions of this early food, as described by Koehler (25), suggest that it is a glandular secretion rather than a regurgitation of predigested honey and pollen from the ventriculus. The chances of larvæ of this age during mass feeding receiving infective material are, therefore, slight.

TABLE VIII.—Ratio of weight in milligrams of unconsumed food in cell to weight of larva at different approximate ages (from Sturtevant (35))

Approximate age (days)	Number of observations	Average weight of food per cell	Average weight of larvæ	Average ratio, food to larva
Egg.....	Several.	Milli-grams None.	Milli-grams a 0. 10	0
1.1.....	33	3. 96	1. 02	3. 88
1.4.....	131	3. 23	1. 36	2. 37
2.1.....	24	9. 10	7. 20	1. 26
2.6.....	35	11. 79	17. 48	. 67
3.0.....	25	5. 05	25. 22	. 20
3.5.....	65	7. 75	63. 46	. 12
4.4.....	17	8. 76	115. 15	. 08

a From Nelson (35).

Soon after the second day, a change in the composition of the food of the larva occurs, accompanied by a change in the method of feeding it by the nurse bees (26). The larva is now fed at approximately the rate at which the food is ingested by it, the demand for food rapidly increasing, accompanied by the great increase in actual body weight, until the time of sealing. The food now contains many entire pollen grains and has a much higher sugar content, nearly 14 per cent, and a relatively lower nitrogenous content (Table III). The principal ingredients are now honey or nectar and pollen. It is well known that honey which is gathered while disease is present in the hive usually carries infection. There is, therefore, a much greater opportunity for infection to occur when the larvæ are being given a food containing unmodified honey as one of its chief ingredients. Furthermore, the constant care of the larvæ during the period of progressive feeding and the large number of nurse bees which visit the cell still further increase the chances of infection being introduced during the period of progressive feeding. It is the young bees in the colony which act as nurses, and these bees are also the ones which clean the hive, so that they are more apt to have infected material on their mouth parts and elsewhere than are old field workers. There can, therefore, be little doubt that it is almost exclusively during the period of progressive feeding that infection normally occurs. Under normal feeding conditions the disease organisms can not develop in the larval intestine until after



feeding has ceased and the sugar-containing food has largely been assimilated, because, with the rate at which the larva is given food containing about 14 per cent sugar, the sugar concentration in the intestine must rapidly increase beyond the 3 to 4 per cent concentration which inhibits growth, so as to prevent the development of the disease in the larva. From Tables IX and X it may be seen that from the second day on, increasing numbers of visits are made to the larva with an increasing amount of time spent in nursing (Table X), so that on the last day of feeding a nursing visit, averaging six seconds in duration, is made approximately every 30 seconds, or an average total during the last 24 hours of 2,855 visits, or 36 per cent of the average 7,858 visits made during the entire feeding period (Table IX). The rapid consumption of this great amount of food, which is supplied in almost a steady stream, is indicated by the fact that the amount of food in the cell with the larva decreases to less than 10 per cent of the weight of the larva just prior to sealing. Even though the exact amount of food consumed may not be known, the actual concentration of sugar in the intestine during this period must rapidly rise, because of the high concentration of sugar in the food and the volume at which it apparently is fed to the larva, to several times the concentration necessary to inhibit the growth of *Bacillus larvae*. There is, as mentioned above, a diluting factor due to the accumulation of undigestible material until the connection is made with the end intestine, but, as shown by the microscopical examination of the intestinal contents, this, because it is largely insoluble, is probably of relatively slight importance in its effect upon the actual sugar concentration in the intestine during the period when inhibition occurs.

TABLE IX.—Relation of nursing to increase in weight of larva <sup>a</sup>

Age (days)	Average weight of larvæ	Average daily increase	Average daily ratio of increase to preceding weight	During active feeding period	
				Number of visits in 24 hours	Average increase in weight per visit per 24 hours
	Gram	Gram			Gram
Egg.....	0.000100				
1.....	.000650	0.000550	5.5	921.6	0.00000597
2.....	.004745	.004095	6.3	833.8	.00000492
3.....	.024626	.019881	4.19	1163.5	.00001709
4.....	.093990	.069364	2.82	2083.7	.00003329
5.....	.146748	.052758	.56	2855.5	.00001848
6.....	.155005	.008257	.06		

<sup>a</sup> From Sturtevant (35) and Lineburg (26).

TABLE X.—Time spent in feeding <sup>a</sup>

Age (days)	Average time spent in nursing per visit per 10 minute period	Average number of visits per 10 minute period	Average number of seconds per visit	Average frequency of visits	Average number of seconds between visits
	Seconds		Seconds	Seconds	Seconds
1.....	20.73	6.40	3.2	93.7	90.5
2.....	5.93	5.79	1.0	103.6	102.6
3.....	11.42	8.08	1.4	74.3	72.9
4.....	41.00	14.47	2.8	41.5	38.7
5.....	118.08	19.83	5.9	30.3	24.4

<sup>a</sup> Calculated from Lineburg (26).

Since it is shown that the concentration of reducing sugars in the larval intestine is usually sufficient to inhibit the growth of *Bacillus larvae* and thus to prevent the manifestation of American foulbrood until after sealing, it is now necessary to explain the rare cases in which advanced stages of the disease are seen in younger coiled larvæ. Such cases are exceedingly rare, except in colonies where almost every cell in the brood combs is filled with a dried scale, and where the bees have deserted the brood-nest because of this diseased material. There can be no doubt that in these cases the earlier manifestation of the disease is due to the fact that in such colonies the progressive feeding of the larvæ is seriously reduced by the fact that the colony has already been depleted in numbers of adult bees. Since there must be a decrease in progressive feeding in such cases, the concentration of reducing sugars in the intestine of the larva is obviously reduced, causing a condition to exist in these intestinal tracts which no longer inhibits the germination and growth of the causative organism. Such rare cases of young larvæ dead of American foulbrood do not, therefore, disprove the theory regarding the time of the development of the disease which has been here set forth, but rather serve as further substantiation of it.

#### THE COCOON-SPINNING STAGE

Sealing usually takes place on about the fifth day, at which time apparently the intestine contains a maximum amount of unassimilated sugar. After sealing occurs and feeding ceases, a different set of factors influence the concentration of sugar in the intestinal contents, so that there is a rapid steady decrease from this time on. The storage of glycogen and fat, however, continues for a short while from the assimilation of reducing sugar. Soon the movements of cocoon spinning and the histolysis of tissues make necessary the utilization of energy stored in form of glycogen and fat, so that the percentage of these substances begins to decrease as the larva loses weight. The emptying of the intestine of fecal material during this period also tends to decrease the sugar in the intestine, so that by the time the cocoon is finished, some time between the seventh and eighth days, the intestine is empty. The larva has straightened out and become quiescent by the eighth day, and all remaining sugar has now been assimilated. It is during this period that, as the concentration of sugar decreases, a point is reached where the growth of the organism can proceed. This apparently occurs when the sugar concentration in the intestine has decreased to about 3 to 4 per cent or less, probably not until some time between the sixth and seventh days. In the cultural experiments it was found that with 2 per cent or less dextrose, vigorous vegetative growth occurs. This vigorous growth requires, however, from 24 to 48 hours to develop, depending somewhat on the amount of initial inoculum. It is probable, as suggested by observations of Berman and Rettger (6), that this sugar in the food furnishes the energy for vegetative growth, while the soluble nitrogenous constituents of the intestinal contents furnish material for cell metabolism, until the time when the organisms have increased in number sufficiently to cause death and are able to invade the tissues of the larva, causing their subsequent decomposition.

#### THE QUIESCENT STAGE

As also is known from long observations of symptoms, the death of the larva and invasion of the body tissues do not in the majority of cases take place until after the larva has at least reached the age of 8 days and has become quiescent. This fact explains the characteristic uniformity of position and appearance of the majority of the larvæ dead from American foulbrood (figs. 7, 8, and 9). There are occasional cases in which death is still further delayed for some reason

until external transformation in form has begun, so that after death the pupa tongue is seen extended and often attached to the upper side wall of the cell in a characteristic manner (fig. 17.) It is possible that in such cases the initial inoculum was smaller than the average, thereby retarding maximum growth of the organism, as was noted in the cultural experiments, and delaying the production of sufficient toxin to kill until this stage of development had been reached.

While the biochemical relations of the bee larva to the disease are seemingly quite adequate to explain the delay in the time when American foulbrood is manifest, there is one other consideration which should be mentioned. The ability of the larva to resist the invasion of the bacteria is a subject on which virtually nothing is known, yet there must be some such ability, as is suggested by the fact that a slight initial inoculum in a colony may not cause the disease to be manifest. At the time when the biochemical conditions are most favorable for the germination and growth of the invading organisms, the larva itself has reached that stage of its development when its internal structure is materially modified by histolysis in advance of pupation, and it must follow almost necessarily that its power of resistance is reduced. The extent to which this factor is involved is, for the time being, purely a matter of speculation.

The variation in the content of reducing sugar of the healthy honeybee larva and the inhibition of the germination and growth of *Bacillus larvae* by a concentration of over 3 or 4 per cent yield an interesting fact concerning the other serious disease of the brood of bees. European foulbrood makes its attack on the bee larva at an earlier stage in its development, while the content of reducing sugars is still high. It must, therefore, be concluded that *Bacillus pluton* has the ability to grow and rapidly to produce toxic substances sufficient to kill the larva in a medium of much higher reducing-sugar content than has *Bacillus larvae*, probably as high as 15 per cent.



FIG. 17.—Decomposed, dried down remains of pupa dead from American foulbrood, showing characteristic tongue attachment to upper wall of cell (White (55))

#### THE EFFECTS OF BACTERIAL METABOLISM IN THE LARVA

There is little unanimity of opinion concerning the effect of dextrose (glucose) upon nitrogen metabolism by various bacteria. Kendall and Walker (24) concluded that the presence of glucose in the medium delays the production of proteolytic enzyme, indicating the "protein-sparing" action of carbohydrates. Fischer (23) believed that proteolytic enzyme is inactivated by glucose, indicated by inhibition of indol formation. Berman and Rettger (6) state that the presence of a carbohydrate in a culture medium may inhibit protein metabolism, depending on the nature of the medium and on the type of the organism as related to hydrogen-ion concentration. DeBord (20) believes that some bacteria destroy glucose without marked increase in the hydrogen-ion concentration and that the rate of production of amino nitrogen or ammonia nitrogen, which may be affected by the presence of carbohydrates, indicates different types of metabolism of bacteria.

The results of the present investigation, although more or less incomplete on this subject, seem to indicate that *Bacillus larvae* has the ability to decompose nitrogenous material in the presence of carbohydrate, since there must be dextrose available until the stored glycogen of the fat body is entirely hydrolyzed.

Many organisms are unable to attack complex protein unless there is some other source of food present, because, according to Berman and Rettger (6), to be

utilizable by bacteria sufficient growth is necessary by which to produce the enzym capable of splitting the complex protein molecule into its simpler amino-acid forms. In the healthy larva at the age when *Bacillus larvae* starts growth in the intestine, the remaining sugar in the intestinal contents and other material of nitrogenous nature in the food is sufficient, as stated earlier, to produce the energy for the initial growth of the organism. By the time the invasion of the tissues by the organisms occurs sufficient proteolytic enzym has been produced to attack the body proteins. Furthermore, the process of histolysis itself, as stated in relation to gelatin liquefaction by ropy material, has probably broken up sufficient of the body proteins to serve as food for bacterial metabolism.

Since *Bacillus larvae* belongs to the spore-forming group of organisms, its processes of metabolism may be similar to those of *Bacillus subtilis*, as described by Berman and Rettger (6), as follows: "The ability of *Bacillus subtilis* to break down protein in the presence of fermentable sugar, and in the absence of an added buffer, may be explained as follows. This organism attacks glucose slowly, and for this reason it is able to produce its proteolytic enzym before the hydrogen-ion concentration reaches a point unfavorable to further growth. When the enzym is thus formed the products of the nitrogen metabolism neutralize the acid, at least in a measure, and the metabolism therefore continues uninterruptedly."

The fact that the production of indol and even of ammonia can be demonstrated, although they may be produced slowly and in small amounts, indicates that even though considerable acid may be produced by the fermentation of the carbohydrate in the food and the hydrolysis of the glycogen, *Bacillus larvae* has putrefactive functions which bring about the formation of sufficient alkaline protein-digestion products from the larval tissues to neutralize this acid production, thereby maintaining the hydrogen-ion concentration at approximately  $P_H=6.8$ .

### SUMMARY AND CONCLUSIONS

1. It has been shown by the work of others that the glycogen and fat content of the bee larva increases in a definite manner for a time and then decreases. In the present work it is shown that the per cent and amount of reducing sugar likewise increase after the third day of larval feeding, but decrease rapidly immediately after feeding has ceased, until by the eighth day no reducing sugar remains in the larva.

2. The presence of reducing sugar can be determined only after the progressive feeding of the larva begins.

3. The best method for extracting reducing sugars from the bee larva was found to be by the use of 50 per cent alcohol and the removal by filtration of the insoluble materials. By this means the interference of glycogen with the action of the reducing solutions is prevented.

4. The constitution of the protein molecule of the normal tissue content of the healthy bee larva is complex, containing, however, among other amino-acids, tryptophan.

5. The best medium for the growth of *Bacillus larvae* so far devised is a yeast-extract agar medium to which sterile egg-yolk suspension has been added. The optimum reaction for cultural growth is  $P_H=6.8$ .

6. Reducing sugars in the culture medium of more than 3 or 4 per cent usually inhibit germination of spores and growth of *Bacillus larvae*. A few spores may germinate at higher concentrations, but the resulting vegetative forms fail to increase in number and show granular disintegration due to autolysis. Less than 2 per cent of reducing sugars seems to stimulate growth of the vegetative forms.

7. The food of the older honeybee larva contains a high percentage of reducing sugar, which is derived from the honey or nectar used in its production. The concentration of reducing sugar in the larval intestine is more than sufficient to inhibit the growth of *Bacillus larvae* until after feeding has ceased. After feeding ceases, the remaining reducing sugar is rapidly assimilated, so that by the seventh day the concentration of sugar has been reduced sufficiently for the active growth of *Bacillus larvae* to occur.

8. The incubation period of *Bacillus larvae* is 24 to 48 hours, so that growth sufficient to kill the larva does not occur until it has completed the spinning of its cocoon and has extended quiescent in the cell, on or after the eighth day, by which time all reducing sugar has disappeared from the larva.

9. The delayed death of the larva in American foulbrood is, therefore, correlated with the inhibiting effect of unassimilated reducing sugar in the intestine upon the germination and growth of *Bacillus larvae*.

10. *Bacillus larvae* has the ability to produce considerable acid, but the hydrogen-ion concentration of the decomposing material is not thereby increased, because of the neutralizing effect of protein decomposition products. The hydrogen-ion concentration of the diseased larva throughout its decay varies only slightly from  $P_H=6.8$ .

11. *Bacillus larvae* not only utilizes reducing sugar for its initial growth, but also completely hydrolyzes the glycogen of the larval body tissues in the process of decomposition.

12. *Bacillus larvae* has the ability to decompose nitrogeous materials, with the formation of amino-acids, indol, and ammonia, but the hydrogen-ion concentration is not decreased by this action, because of the concomitant production of acids from carbohydrates.

13. *Bacillus larvae* apparently has no action on fat.

14. The biochemical data herein presented for the first time explain the remarkable characteristics of American foulbrood, which were left entirely unexplained from observations on etiology alone.

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BRITTLE STRAW AND OTHER ABNORMALITIES IN RYE<sup>1</sup>

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In America rye is of minor importance as a grain crop; consequently there has been relatively little time devoted to its improvement. However, rye combines many desirable qualities such as winter hardiness, wide soil adaptation, quick growth, and good yield. It seems probable, therefore, that it will eventually find wider favor in this country.

Two general methods of improvement of rye are employed at the Minnesota Agricultural Experiment Station, namely, bulk selection for purity of seed-color and for vigor of the plants; and selection in self-fertilized lines. Minnesota No. 2 rye, which originated at Minnesota as a result of selection for winter hardy plants in Swedish rye, has been used for all of the inbreeding work. Since rye is normally cross-fertilized, commercial varieties are ordinarily heterogeneous mixtures for seed color and plant type. Both of these methods of breeding seek the isolation of vigorous uniform types, and hence may be considered respectively as broad and narrow types of inbreeding.

One of the interesting features of these studies at Minnesota is the occurrence of certain types of abnormalities, in inbred strains. Since the normal type is usually dominant when in the heterozygous condition such abnormalities are seen only rarely in a commercial variety. Inbreeding, either by selection or by self-fertilization, brings about a recombination of the recessive abnormality factors and results in the appearance of abnormal plants in the inbred strains. This is of the utmost importance since it gives the plant breeder an opportunity to isolate vigorous desirable types and to discard the undesirable ones. It is the purpose of this paper to present some of the data at hand regarding these abnormalities, with particular reference to one called "brittle" on account of the ease with which the straw breaks.

Chlorophyll deficiency is of common occurrence as an abnormality in inbred strains of rye. In 1922, white or "albino" seedlings were found in nearly 25 per cent of the self-fertilized strains and in more than 50 per cent of the strains originated by bulk selection, as shown in Table I.

TABLE I.—*Occurrence of chlorophyll-deficient abnormalities in inbred strains and in normal Minnesota No. 2 rye, in 1922*

	Strains isolated by bulk selection	Self-fertilized strains	Normal Minnesota No. 2 rye
Number of strains in the rye nursery.....	18	200	.....
Number of strains segregating for white seedlings.....	13	45	.....
Range of percentage of white seedlings to normal green seedlings in the inbred strains.....	0.4-32.4	4-60	0.04
Number of strains segregating for other types of chlorophyll abnormalities.....	3	23	.....

<sup>1</sup> Received for publication Nov. 22, 1923.

TABLE II.—Occurrence of “brittle” plants in selfed-strains of rye, in 1922

1922 culture number	Years selfed	Normal plants	Brittle plants
R 11.....	1, D-P <sup>a</sup> .....	28	2
R 19.....	1, D-P.....	17	5
R 36.....	1.....	6	3
R 37.....	1.....	5	1
R 39.....	D-P <sup>b</sup> .....	12	6
R 41.....	1.....	16	2
R 43.....	D-P.....	14	7
Total.....	.....	98	26

<sup>a</sup> 1, D-P=one year selfed followed by D-P.  
<sup>b</sup> D-P=intercross, that is, two heads on different plants in the same culture bagged together.

Chemical analysis of the plants was undertaken to determine the cause of brittleness. Samples of brittle rye and normal rye were put up while green in 85 per cent ethyl alcohol containing sufficient Ca(CO<sub>3</sub>)<sub>2</sub> to neutralize any acids.

TABLE III.—Analysis of normal and brittle rye culms

Sample	Dry weight	Per cent ash	Sugar			Pentosans	Starch	Total pectin	Crude (dry basis)	Fiber (wet basis)
			Red	Sucrose	Total					
	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>
Brittle rye.....	27.03	5.49	7.09	6.47	13.57	34.34	0.78	0.234	13.50	3.81
Do.....		5.44	7.12	6.50	13.60	33.35	.81	.242	14.75	
Normal rye.....	40.58	4.91	5.60	4.63	10.26	25.40	.98	.20	32.21	13.04
Do.....		4.85	5.67	4.79	10.42	24.60	1.04	.20	32.10	

In Table III a comparison of the principal chemical constituents which may be associated with the production of brittle or healthy plants is given. The percentage of dry weight of the brittle rye and of the normal rye shows quite an appreciable difference. The brittle rye contains the lower percentage of dry material. This suggests a high content of colloid with high water-holding capacity, a condition which is indicated by the greater pentosan content of the brittle plants. Culms that had a high water content or were turgid would have a tendency to snap off easily as the brittle plants do.

The crude fiber determination shows the relative amount of skeletal material of the plants, particularly cellulose and lignin, which are responsible for the strength of the culm. There is a ratio of 32.15 per cent crude fiber in the normal plants to 14.13 per cent in the brittle plants, comparison being made on the dry weight basis. When calculated on the wet weight basis, the ratio of crude fiber of the normal rye to the brittle is 13.04 to 3.81 per cent. The proportion of crude fiber on the wet weight basis is the proportion we would find in the stalk in the field, and this extreme difference in crude fiber may explain to a great extent the difference in the strength of the stems.

The pentosan content of the brittle rye is higher than that of the normal rye on the dry weight basis, but is almost the same on the wet weight basis. The pentosan material being chiefly hydrophilic colloids does not aid appreciably in the skeletal make-up or strength of the stalk, except as it enters into the composition of the ligno celluloses. This higher percentage of pentosan in the brittle rye may explain the higher water content of the brittle stalks, when compared with the normal ones.

The ash, starch, and pectin contents are not very different in the two samples. The high sugar content and higher pentosan content coincident with low crude fiber in the brittle plants indicate that the carbohydrates do not contribute to wall-forming material in the normal way.

The methods used in the analysis of ash, starch, crude fiber, and pentosan were taken from the "Methods of Analysis of the Association of Official Agricultural Chemists."<sup>2</sup> The method used for the pectin determination was that

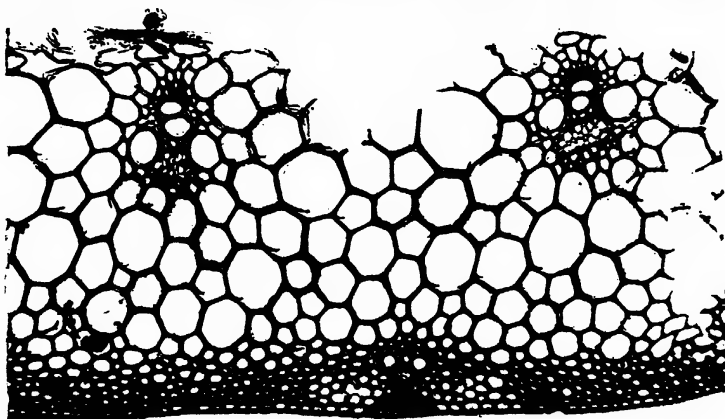


FIG. 1.—Cross section of normal rye stem.  $\times 80$

reported by Carré and Haynes.<sup>3</sup> The sugars were determined by the picric acid method.<sup>4</sup>

Histological and microchemical examinations were made along with the chemical analysis. The microscopic appearance of cross sections of brittle and normal rye stems was quite different, as is shown in figures 1 and 2. While the depth of the sclerenchyma layer at the periphery of the stem was practically the same in the two samples, the thickness of the cell walls was strikingly less

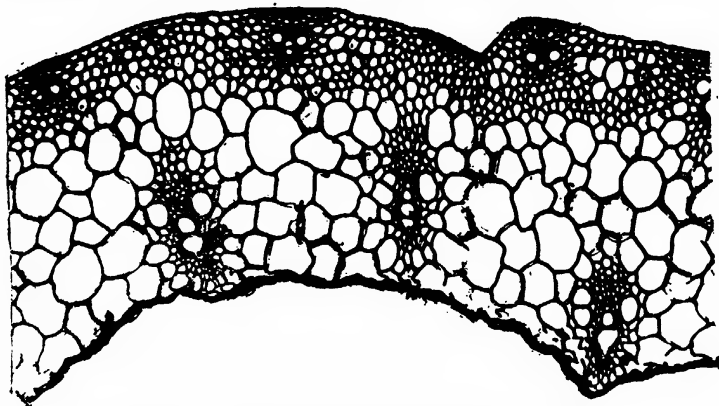


FIG. 2.—Cross section of "brittle" rye stem.  $\times 80$

in the brittle rye, as indicated in Table IV. Garber and Olsen found a similar condition associated with lodging in oats and rye.<sup>5</sup>

<sup>2</sup> ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. As compiled by the Committee on Revision of Methods. Revised to Nov. 1, 1919. 417 p., illus. Washington, D. C. 1920.

<sup>3</sup> CARRÉ, M. H., and HAYNES, D. THE ESTIMATION OF PECTIN AS CALCIUM PECTATE AND THE APPLICATION OF THIS METHOD TO THE DETERMINATION OF THE SOLUBLE PECTIN IN APPLES. *Biochem. Jour.* 16: 60-69. 1922.

<sup>4</sup> LEWIS, R. C., and BENEDICT, S. R. A METHOD FOR THE ESTIMATION OF SUGAR IN SMALL QUANTITIES OF BLOOD. *Jour. Biol. Chem.* 20: 61-72. 1915. (Modified 1923 by Willaman and Davison.)

<sup>5</sup> GARBER, R. J., and OLSEN, P. J. A STUDY OF THE RELATION OF SOME MORPHOLOGICAL CHARACTERS TO LODGING IN CEREALS. *Jour. Amer. Soc. Agron.* 11: 173-186, illus. 1919.

TABLE IV.—*Thickness of the cell wall in brittle and normal rye*

	Brittle rye	Normal rye
Epidermis:	<i>Microns</i>	<i>Microns</i>
External wall.....	2.4-2.9	5.3
Side wall.....	.9	3.3
Sclerenchyma.....	2.8	9.6
Vascular bundle:		
Sheath.....	.4-1.9	4.8
Pitted vessel.....	1.4	2.4
Spiral vessel.....	3.2-4.8	4.8
Pith cell.....	.4-.9	2.4

The data recorded in this table corresponds well with the chemical estimation of crude fiber. Besides thickness of wall, the size of the cells is a factor determining the proportion of crude fiber.

Microchemical examination, using phloroglucin—HCl, showed lignin throughout the walls of both brittle and normal rye, except in the phloem and xylem parenchyma of the fibro-vascular bundles. Differences were in degree rather than in extent of lignification. It was not found practicable to attempt to distinguish between pentosans in the wall and the protoplast, because pentoses demonstrated in the vacuole could easily have diffused in from the hydrolyzed pentosans of the wall.

#### SUMMARY

Brittleness of straw, chlorophyll deficiency, male sterility, and crinkled awns were found as abnormalities in inbred strains of Minnesota No. 2 rye.

Chemical analyses show a low percentage of crude fiber (14 per cent) and high pentosan (34 per cent) content in brittle straw compared with a crude fiber content of 32 per cent and pentosan content of 25 per cent on a dry-weight basis in healthy rye plants. Brittle plants have a high moisture content correlated with high pentosan content. The differences in starch, pectin, ash, and sugar content of normal and brittle rye plants are not great in amount.

Evidently the carbohydrates in the brittle plants are not normally transformed into cellulose and wall-forming substances designated as crude fiber, but accumulate as pentosans.

The greater thickness of cell wall in the normal rye accounts in part for its greater strength as compared with brittle rye straw.

The amount of lignin in normal rye straw greatly exceeds that in brittle rye

# RELATION BETWEEN TOXICITY OF COTTONSEED AND ITS GOSSYPOL CONTENT <sup>1</sup>

By ERICH W. SCHWARTZE, *Pharmacologist in Charge, Pharmacological Laboratory*, and CARL L. ALSBERG, *formerly Chief, Bureau of Chemistry, United States Department of Agriculture*

## INTRODUCTION

Analysis of many samples of cottonseed (9)<sup>2</sup> has shown that the gossypol content tends to vary with the place where the seed is grown. A positive correlation between the gossypol content of the seed and its oil content was also found. The fact that the seeds which were low in gossypol were grown in the Southwest where cottonseed poisoning is uncommon supports the hypothesis that the tendency toward a regional variation of the gossypol content of the seed accounts for the tendency toward a regional distribution of cottonseed poisoning. The experiments herein reported deal with this hypothesis. The toxicity of four samples of raw cottonseed kernels having extremely high and moderately high gossypol contents, as observed in animal experiments, is compared with their toxicity as estimated from their gossypol contents. It was thought that if the toxicities in each case should prove to be similar or identical, such parallelism would go far to indicate that gossypol is the cause of cottonseed poisoning (1, 2).

## EXPERIMENTAL MATERIAL AND METHODS

### KERNELS (MEATS) AS THE SOURCE OF POISON

The kernels (meats), practically free from hulls and lint, rather than the commercial meal, were used in order to eliminate any effect upon the toxicity of the seed resulting from the heat and pressure employed in the process of expressing oil. Moreover, the method of estimating gossypol in cottonseed press cake has not been sufficiently developed. It is believed that a considerable quantity of the decomposition products of gossypol is present in the meal. Concerning their pharmacological action little is known.

### PREPARATION OF GOSSYPOL

The gossypol used in these experiments was isolated and purified as the "acetate" by the method of Carruth (3). Two large lots were prepared from different samples of gin-run seed and a third was prepared from a mixture of select and gin-run seed. Had the requirements of the experiments been fully anticipated in the beginning, a single lot would have been prepared. Each preparation was examined and identified by E. T. Wherry, crystallographer of the Bureau of Chemistry. Their decomposition points were also determined (176°-180° C.). The preparations fed were freed from the acetic acid by washing an ether solution of gossypol acetate three times with water.<sup>3</sup> The ether solution was poured into a dish of water and evaporated on the steam bath. The crusts of gossypol were dried in the air and later ground.

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<sup>1</sup> Received for publication, Mar. 6, 1924.

<sup>2</sup> Reference is made by number (*italic*) to "Literature cited," pp. 188-189.

<sup>3</sup> Gossypol "acetate" seems to be unstable in ether solution. The precautions to free the gossypol from acetic acid may have been superfluous, as hydrolysis may occur also in other solvents, such as fat or oil.

## PREPARATION OF EXTRACTS OF COTTONSEED KERNELS

The cottonseed kernels were extracted in a Soxhlet extraction apparatus until no significant yellow color was imparted to the ether which bathed the thimble and contents, a procedure that required from 18 to 48 hours. The ether was evaporated from the extract with the aid of heat, and the residual oil, diluted with peanut oil, was used for the experiments in the manner described.

## DIETS

In the feeding tests four diets were used: Control peanut meal; gossypol; raw cottonseed kernel; and control ether-extracted cottonseed kernel. The first three contained an abundance of peanut meal, an adequate source of protein. All diets contained an abundance of butterfat as a source of fat-soluble vitamin, an abundance of milk powder supplying protein, and an adequate salt mixture. They contained roughage in the form of agar-agar, except those containing the greatest quantity of each variety of raw cottonseed kernels, which were used only for a few of the shorter feeding experiments.

The control peanut meal diet and the gossypol diet differed only in the presence of gossypol. The control ether-extracted cottonseed kernel diet was the same as the other diets, except that it contained ether-extracted cottonseed kernels and additional fat instead of peanut meal or whole cottonseed kernels. The gossypol and fat had been removed from the cottonseed kernels by ether extraction in the course of quantitative gossypol analyses formerly made (9). The raw cottonseed kernel diet was the same as the control diet except that part of the peanut meal and fat had been replaced by different varieties of raw cottonseed kernels in varying proportions. The composition of the various kernels and diets is given in Tables II and III. In each case the diet contains all the ingredients essential for normal growth.

TABLE I.—*Ether extracts injected intraperitoneally*

Variety extracted	Cottonseed		Ether extract		Gossypol in diluted extract for injection
	Quantity extracted	Gossypol content	Gossypol content	Peanut oil added to make—	
	Gm.	Per cent	Mgm.	Cc.	Per cent
Trice.....	54.6	0.411	224	50	0.449
Lone Star.....	42.2	.518	218	50	.437
Durango.....	26.2	.984	257	50	.514
Egyptian.....	19.2	1.180	227	50	.454

TABLE II.—*Composition of cottonseed kernels and other material*

Material	Ether extract	Nitrogen	Moisture	Gossypol
	Per cent	Per cent	Per cent	Per cent
Trice kernels.....	28.87	6.42	6.12	0.416
Lone Star kernels.....	33.40	6.40	-----	.518
Durango kernels.....	38.97	4.94	5.93	.984
Egyptian kernels.....	36.68	4.73	5.66	1.180
Ether-extracted cottonseed kernels I <sup>a</sup> .....	b 0	9.01	10.92	b 0
Ether-extracted cottonseed kernels II <sup>a</sup> .....	b 0	8.59	-----	b 0
Peanut meal I.....	(c)	8.30	-----	0
Peanut meal II.....	10.33	8.11	6.59	0

<sup>a</sup> Composite samples resulting from the chemical analyses reported by Schwartze and Alsberg (9).

<sup>b</sup> Gossypol and fat were removed by ether extraction. A trace of each was probably present.

<sup>c</sup> Only a few experiments were made with this sample. Ether extract was assumed to be 10.33 per cent in making the calculations.

TABLE III.—Composition of diets

Diet	Ingredients						Composition		
	Cotton-seed kernels	Peanut meal	Ether-extracted cottonseed kernels	Butter-fat	Refined oil <sup>a</sup>	Milk powder	Gossypol	Protein <sup>b</sup>	Fat <sup>b</sup>
	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.
Peanut I <sup>c</sup>		40.0		25.0		20.0	0.0	22.8	34.6
Peanut II <sup>c</sup>		40.0		25.0		20.0	.0	23.3	34.6
Ether-extracted cottonseed kernels Ia <sup>c</sup>			35.0	30.0		20.0	.0	21.0	35.5
Ether-extracted cottonseed kernels Ib <sup>c</sup>			38.0	25.0	2.0	20.0	.0	22.0	32.5
Ether-extracted cottonseed kernels II <sup>c</sup>			33.6	17.0	14.4	20.0	.0	20.0	36.9
Gossypol (0.0675) <sup>c</sup>		40.0		25.0		20.0	.065	23.3	34.6
Gossypol (0.1350) <sup>c</sup>		40.0		25.0		20.0	.135	to	
Gossypol (0.2250) <sup>c</sup>		40.0		25.0		20.0	.225	22.8	
Trice (15) <sup>c</sup>	15.0	30.0		20.0		20.0	.062	24.0	32.9
Lone Star (13) <sup>c</sup>	13.0	31.0		21.0		20.0	.067	23.7	34.0
Durango (7.5) <sup>c</sup>	7.5	36.5		21.0		20.0	.074	23.8	33.2
Egyptian (6) <sup>c</sup>	6.0	37.0		22.0		20.0	.071	23.4	33.5
Trice (30) <sup>c</sup>	30.0	20.0		15.0		20.0	.123	24.7	31.2
Lone Star (26) <sup>c</sup>	26.0	20.0		19.0		20.0	.135	23.3	35.3
Durango (15) <sup>c</sup>	15.0	30.0		20.0		20.0	.148	22.8	34.5
Egyptian (12) <sup>c</sup>	12.0	32.0		21.0		20.0	.142	22.7	34.2
Trice (54.6) <sup>d</sup>	54.6	6.7		13.7		20.0	.224	27.3	35.7
Lone Star (42.2) <sup>e</sup>	42.2	18.9		13.7	.2	20.0	.218	28.5	37.8
Durango (26.2) <sup>e</sup>	26.2	31.3		13.7	3.8	20.0	.257	26.4	36.5
Egyptian (19.2) <sup>e</sup>	19.2	36.5		13.7	5.6	20.0	.227	26.7	35.6

<sup>a</sup> Refined but not deodorized cottonseed oil made in the Oil, Fat, and Wax Laboratory of the Bureau of Chemistry.

<sup>b</sup> The factor 5.5, used for calculating the protein of peanut meal and of cottonseed, is sufficiently accurate for these experiments. Commercial samples of whole milk powder have been assumed to contain approximately 25 per cent of protein and 27.5 per cent of fat.

<sup>c</sup> Corn starch (9 per cent), agar-agar (3 per cent), and salt mixture IV (3 per cent) (Osborne and Mendel, 7, p. 374) were supplied in these diets.

<sup>d</sup> This diet was not used, as the supply of seed became exhausted.

<sup>e</sup> These diets contained 2 per cent of corn starch and 3 per cent of salt mixture IV. No agar-agar was added.

Since gossypol is not evenly distributed in the seeds, but is found in the “gland dots” of the kernels (10), it seemed advisable to subject the kernels intended for feeding to a treatment which would uniformly distribute the gossypol through them. Accordingly, the kernels were ground and mixed with peanut meal. The mixture of peanut meal and finely ground kernels was then treated with ether. After having been warmed sufficiently, with constant stirring, the ether was evaporated on the steam bath, and the resulting dry lumpy meal was sifted and exposed for 18 hours to the atmosphere in thin layers on a dry glass plate. No odor of ether was apparent, and the food made from the meal was as acceptable to animals as that containing comparable quantities of gossypol.

METHODS OF ADMINISTRATION

Methods of administering the cottonseed kernels have been described. In the experiments in which free gossypol was added to the diet, it was incorporated in the butter fat used in the preparation of the diet. Possibly the gossypol entered into combination with some one of the substances present in butter, for in the more concentrated butter-fat solutions used in another series of experiments the gossypol crystallized out. It is not known whether or not gossypol is loosely combined with an organic acid in the cottonseed. In several feeding and intra-peritoneal injection experiments, however, no difference in toxicity between gossypol and gossypol acetate was observed. It is therefore reasonable to con-

clude that any loose compound that may have been formed in butter would not materially influence the results of these experiments. Whatever the form in which the gossypol eventually may have been found and whatever its source, care was taken that it should be distributed evenly throughout the medium and that the state of subdivision should be as fine as the condition of the preparation would permit.

Whenever the gossypol was not administered *per os* it was given dissolved in peanut oil. Published data on the chemistry of gossypol, as well as the authors' experience, indicate that the best way to prepare this substance for intraperitoneal administration is to dissolve it in oil. The rate of oxidation as judged by color changes is much less in this medium than in aqueous alkali or in alcohol. Where the object was to determine the toxicity of a given sample of cottonseed rather than the toxicity of gossypol itself in the pure state, the ether extracts of the seeds to be tested were used. Their method of preparation has been described. It is obvious that for these experiments the proper procedure was to use the whole ether extract rather than pure gossypol prepared from a given sample of seed. The oil solution of gossypol and the oily residue obtained in the ether extraction of cottonseed kernels were always administered intraperitoneally, the most suitable way to inject an oil solution.

#### ANIMALS USED

Under the conditions obtaining in these experiments, rats appear somewhat unsusceptible to gossypol intoxication and do not show some of the typical symptoms of cottonseed poisoning found in certain farm animals. Because of the convenience of performing a large number of experiments simultaneously, however, they were used. The results establish the relation of the toxicity of different samples of seed to one another and to pure gossypol. All animals were of the same sturdy stock, reared in the laboratory. Variability was thus reduced to a minimum.

A few grown mice and a number of grown rats were used. In the early feeding experiments, however, growing rats proved best for the tests, so that the discussion is confined chiefly to experiments with them.

#### RESULTS OF EXPERIMENTS

##### INTRAPERITONEAL INJECTION OF PURE GOSSYPOL AND OF THE ETHER EXTRACT OF DIFFERENT VARIETIES OF COTTONSEED KERNELS

Gossypol was injected intraperitoneally because it is readily absorbed from an oil in the peritoneal cavity.<sup>4</sup> Gossypol is more potent by this route than by oral or subcutaneous administration. Apparently the injected gossypol leaves the oil and some of it is dissolved in serum exudate. The oral administration of gossypol dissolved in oil is followed by diarrhea and also by paralysis of the intestine and stomach musculature. From these phenomena it follows that the dosage varies with the degree to which the local phenomena are superimposed upon one another. In one case gossypol was recovered in the oil found in the stomach, the oil having been given by a stomach tube seven days before death. Absorption from the subcutaneous tissue is very poor. The intravenous administration of an emulsion of an oil solution of gossypol was not attempted because with the relatively small quantities of gossypol available no means were at hand for the preparation of an emulsion sufficiently fine to preclude fat embolism.

<sup>4</sup> Later experiments have shown that cottonseed oil becomes emulsified slowly, whereas peanut oil does not. No deleterious symptoms from the injection of pure cottonseed oil or peanut oil were observed during the lifetime of the animals.



The results of the intraperitoneal tests are given in Table IV. With a few exceptions, the percentage of gossypol dissolved in oil was 0.45 per cent. The minimum lethal dose is about 20 mgm. and the largest survived dose is about 50 mgm.<sup>5</sup> Smaller rats seem to require larger doses than larger animals. Individual differences in susceptibility to cottonseed poisoning between animals of the same age have been observed by other investigators. An effort was made to eliminate these as far as possible in the rat studies by using only animals that had been reared in the laboratory under standard conditions. Deaths from the smaller doses are delayed and to a great extent give the impression that the effect of starvation is superimposed upon an intoxication factor. Although 45 to 50 mgm. per kilo may be regarded as the practically certain fatal dose, all deaths did not occur within 24 hours as they did when larger doses were administered.

Tests with the ether extract of Egyptian cottonseed kernels indicate that these kernels are as toxic as the gossypol content determined by analysis would show them to be. The results in this case are even more uniform than those in the tests in which pure gossypol was used.

Tests with the ether extract of Durango cottonseed kernels also indicate that these kernels are as toxic as the gossypol content determined by analysis would show them to be. At the time these experiments were made, the gossypol content of the extract was underestimated by about 10 per cent. This explains why the dosage reported is slightly larger than in the case of the Egyptian seed extract.

TABLE IV.—*Toxicity to rats of gossypol and of volumetric solutions of ether extracts of cottonseed of which the gossypol content has been estimated (oil solution injected intraperitoneally)*

GOSSYPOL											
Num- ber of rats	Weight	Dose of gossypol (calcu- lated)	Fatalities							Survived	Remarks
			First day	Sec- ond day	Third day	Fourth day	Fifth day	Sixth day	Total		
	<i>Gm.</i>	<i>Mgm. per Kilo</i>									
4-----	241-278	10-17	0	0	0	0	0	0	0	4	
1-----	244	18. 5-20	0	1	0	0	0	0	1	1	
5♀-----	100-215	18	0	0	0	0	0	0	0	5	
4♀-----	177-205	18	0	0	3				3	1	All pregnant.
5♂-----	131-202	22. 5	0	0	1	0	0	3	4	1	All refused food.
2-----	181-265	25-30	0	2	0	0	0	0	0	2	One pregnant.
5♂-----	125-175	31. 5	0	2	3				5	0	
3-----	198-254	33-35	0	2	0	0	0	0	2	1	
3-----	70-80	36	0	0	0	0	0	0	0	3	Killed on 12th day.
5♂-----	146-177	45	2	2	1				5	0	
2-----	68-78	45	0	0	0	1	1		2	0	
2-----	159-187	45-49	1	1					2	0	
1-----	189	49	0	0	0	0	0	0	0	1	
7-----	64-195	55-90	7						7	0	

EGYPTIAN COTTONSEED											
5♂-----	135-165	18	0	0	0	0	0	0	0	5	One dead on 9th day.
5♂-----	145-177	45	5						5	0	4 died within 16 hours.

<sup>5</sup> Experience with this intraperitoneal fixed-oil method, using a large number of fat-soluble dyes, also reveals a variation in the toxicity of individual substances. This must be controlled by using a sufficient number of animals and making comparisons of the toxicity ranges obtained.

TABLE IV.—Toxicity to rats of gossypol and of volumetric solutions of ether extracts of cottonseed of which the gossypol content has been estimated (oil solution injected intraperitoneally)—Continued

DURANGO COTTONSEED											
Number of rats	Weight	Dose of gossypol (calculated)	Fatalities							Survived	Remarks
			First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Total		
	Gm.	Mgm. per Kilo									
5 ♀	125-130	15.3	0	0	0	0	0	0	0	5	
5 ♂	145-175	20.7	1	0	0	0	0	0	1	4	
5 ♂	140-210	20.7	3	0	0	0	0	0	3	2	1 died on 12th day.
5 ♂	140-177	51.3	4	0	0	0	0	0	4	1	4 died within 16 hours.
LONE STAR COTTONSEED											
5	112-222	17.5	0	0	0	0	0	0	0	5	All lived.
5 ♀	130-155	43.7	0	0	0	0	0	0	0	5	2 died later
5 ♀	210-260	65	3	0	1	0	0	0	4	1	
TRICE COTTONSEED											
5 ♂	195-215	18	0	0	0	0	0	0	0	5	1 died on 15th day.
5 ♀	132-147	45	0	3	1	0	0	0	4	1	1 killed on 10th day.
5	127-145	58.5	3	0	0	0	0	0	3	2	2 killed on 10th day.

The tests with the ether extract of the Lone Star cottonseed kernels indicate that their toxicity is not so great as might have been inferred from their gossypol content as determined by analysis. The best estimate that can be made is that the seeds are only about two-thirds as toxic.

The tests made with the ether extract of the Trice cottonseed kernels indicate that the seed is slightly less toxic than indicated by the gossypol content as determined by analysis, the best estimate being about three-fourths of the theoretical value.

The Durango and Egyptian seeds which contained the larger quantities of gossypol proved as toxic as predicted on the basis of the chemical analysis, whereas Lone Star and Trice seeds were less toxic than predicted. Had the toxicity been underestimated instead of overestimated, evidence that some factor other than the gossypol was involved would have been furnished. This slight deficiency of toxicity, however, is not inconsistent with the gossypol hypothesis of cottonseed poisoning. This substance is not entirely stable, and a small quantity could easily have been oxidized after picking the seed. Nothing concerning the treatment, fumigation, or storage of these samples of seed was ascertained. Moreover, the extracts represent crude gossypol, and the method of analysis includes the estimation of a small quantity of some substance other than gossypol, presumably a decomposition product of gossypol.

A comparison of the results in Table I with those in Table IV shows that the order of toxicity of the seed used is the same as that indicated by the chemical analysis, but that the Egyptian seed, instead of being about three times as toxic as Trice cottonseed, is about four times as toxic. Analogous relations exist for Lone Star seed. These somewhat aberrant results may be within the possible range of error of the analytical method.

## FEEDING EXPERIMENTS

## CONTROL DIET OF PEANUT MEAL AND CONTROL DIET OF ETHER-EXTRACTED COTTONSEED KERNELS

The growth curves of the rats fed the control diets are given in figures 1 and 2. The charts have been compared with 50 gm. body weight as a base line or arbitrary starting point, since weight rather than age was the criterion of comparison. The average rate of growth for males and for females has also been

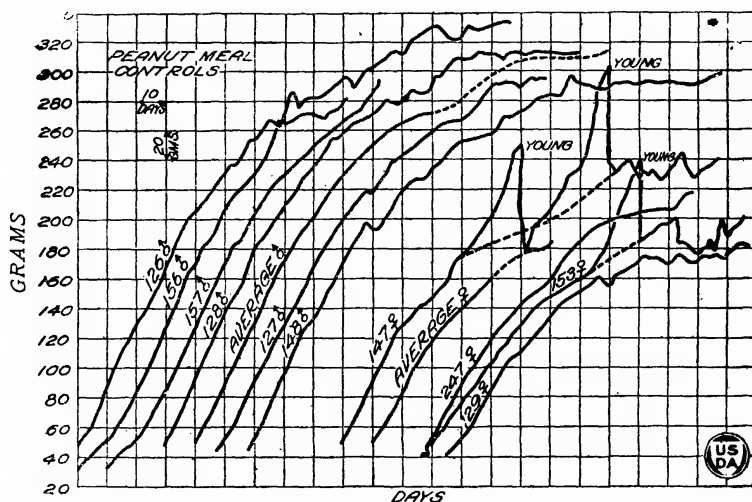


FIG. 1.—Growth of male and female rats on control peanut meal diet. Broken line on “average” curve represents estimated average rate of growth from fewer observations than for unbroken line. Peaks on female growth curves labeled “young” represent weight fluctuation due to pregnancy and birth of young. Broken line on curves of female rats represents hypothetical course of weight variation if pregnancy had not occurred

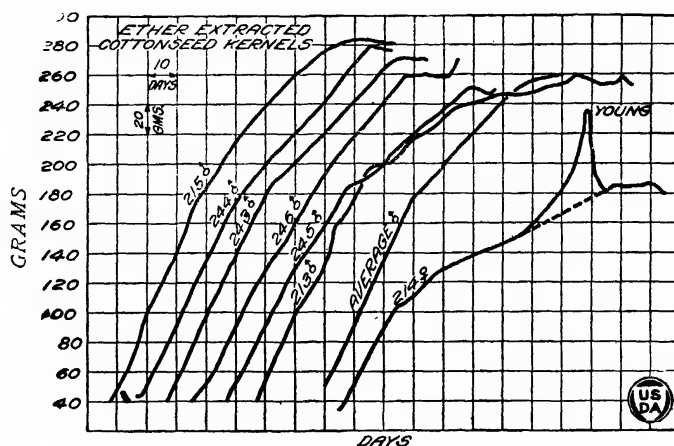


FIG. 2.—Growth of male and female rats on ether-extracted cottonseed kernel diet. No. 213, 214, and 215 were fed 30 per cent butter fat and the remainder 17 per cent butter fat. Broken line on one curve represents estimated weight if pregnancy had not occurred

computed and is shown on the charts. The rate of growth was much faster than that usually recorded in the nutrition literature and seemed to be associated with the sturdiness of the rats and with the high protein diet. The rapidity of growth necessitated the selection of 10 days for each unit on the abscissa instead of the conventional 20-day period, in order to keep the growth curves in the neighborhood of the 45-degree line.

Eighty days after the 50-gram body weight had been attained, the weight of the six male rats fed peanut meal ranged from 255 to 284 gm. with 273 gm. as an average. On the fortieth day the range for the four female rats was 147 to 174 gm., with an average of 157 gm.

The range of weights for the six males receiving the ether-extracted cottonseed kernel diet was 245 to 282 gm., with an average of 259 gm. on the eightieth day. The different butter fat quantities fed made no apparent difference in these few experiments. Therefore the growth curves have been arranged together. The maximum range and average body weight are only slightly below those for the males on the peanut meal diet, and appear to be within the limits of experimental error when so few experiments are used. The growth curve of the single female rat does not differ greatly from the rate of growth of the female rats on the peanut meal diet. It is 10 gm. below the lowest weight on the peanut diet. The bulkiness of the ether-extracted cottonseed kernel diet probably accounts for the very slightly smaller average daily intake of this diet (not shown on the charts), in which case, other things being equal, a slightly less rapid growth was to be expected. According to the literature, the utilization of cottonseed protein in animal feeding is not as complete as the utilization of protein in many other kinds of feed. However, it has been shown in the Protein Investigation Labora-

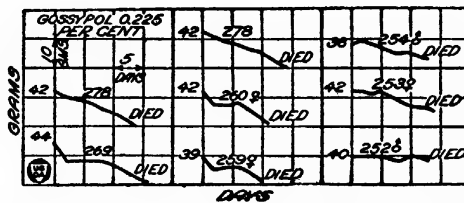


FIG. 3.—Effect on young rats of diet containing 0.225 per cent gossypol. Figure at left of curve indicates the weight at the beginning of the experiment; figure above the curve, the laboratory number of the animal. Because of short duration of experiments chart has been drawn on a scale twice that used in preceding and succeeding charts

tory of the Bureau of Chemistry that isolated cottonseed protein is as well digested *in vitro* as casein and arachin, the protein of the peanut (5).

The conclusion to be drawn from these experiments is that for the purpose of this study there was no significant difference in the rate of growth of rats fed the peanut meal diet and those fed the ether-extracted cottonseed kernel diet.

#### CONTROL DIET CONTAINING ADDED GOSSYPOL

That the diet was totally adequate has been shown. The growth curves in figure 3 show that young rats used in these tests failed to grow and eventually died on a diet containing 0.225 per cent of gossypol. On a diet containing 0.18 per cent two rats died rather soon, while two lived for some time (fig. 4). Recovery to some extent was begun when the diet was changed to the control food. From these few tests it is impossible to say with certainty that the subsequent decline on the control diet was connected with the previous gossypol feeding. From other experiments, however, it would seem that after the removal of gossypol from the diet, animals do not always recover their full health or normal growth rate. It is not certain in any of these tests that some of the results may not have been due to loss of appetite.

The rates of growth of rats upon diets containing 0.045, 0.0675, 0.09, and 0.135 per cent of gossypol are given in figures 5 to 9.

Only two experiments with a concentration of 0.045 per cent of gossypol were made. These were upon male rats. One rat appeared to have grown normally. The other was only slightly inferior to the rats in the control diet. On the eightieth day the average body weight of the two rats on the diet was only about 3 gm. below the average of the male rats fed ether-extracted cottonseed kernels. The effect of this concentration of gossypol, therefore, if deleterious, is apparently too slight to be shown under the conditions of the experiments.

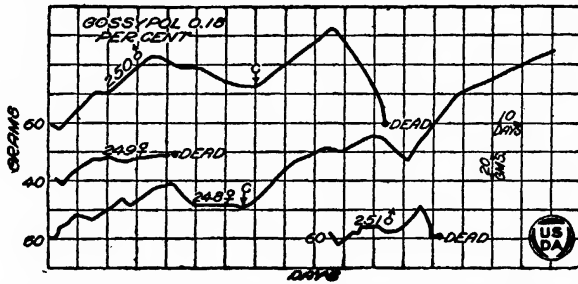


FIG. 4.—Effect of 0.18 per cent gossypol in the diet. Figures at the left of each curve indicate the weight at the beginning of the experiments, and that above the laboratory number of animal. "C" shows a change to control peanut meal diet

Upon a diet containing 0.0675 per cent of gossypol some retardation of rate of growth, as well as a slight tendency toward the attainment of a lower absolute degree of growth, was observed, as shown in the curves of body-weight averages. Some animals, however, made as good a showing as the poorest of the control rats. On the eightieth day the average weight of male rats was 238 gm. or 35 gm. less than that of the control rats on the peanut diet, and 21 gm. less than that of the rats on the diet containing ether-extracted cottonseed kernels. On the fortieth day the female rats averaged 134 gm., as compared with 137 gm. for those receiving the control diet. The growth curves of four rats (two males, No. 222 and 225, and two females, 223 and 224), the offspring of females reared on the

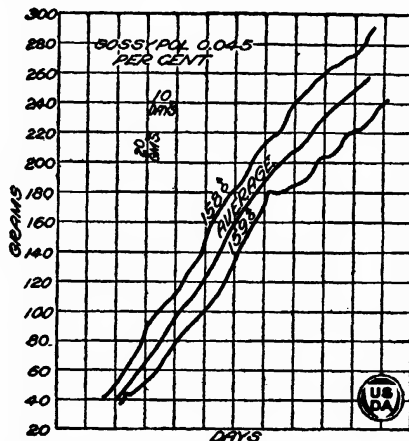


FIG. 5.—Effect of 0.045 per cent gossypol in the diet

diet containing 0.0675 per cent of gossypol and of the males reared upon the control diet, show that the rate of growth was below the average of that attained by the first generation fed upon the 0.0675 per cent gossypol diet, but was as good as that attained by the poorest on such a diet. It is evident, therefore, that the concentration of gossypol in the diet used in these experiments is close to the threshold value for toxicity under the conditions of the experiments. Whether or not it has any effect upon succeeding generations has not yet been determined.

A concentration of 0.09 per cent gossypol, as shown individually in four out of six experiments, produced a more marked retardation of growth. The result of

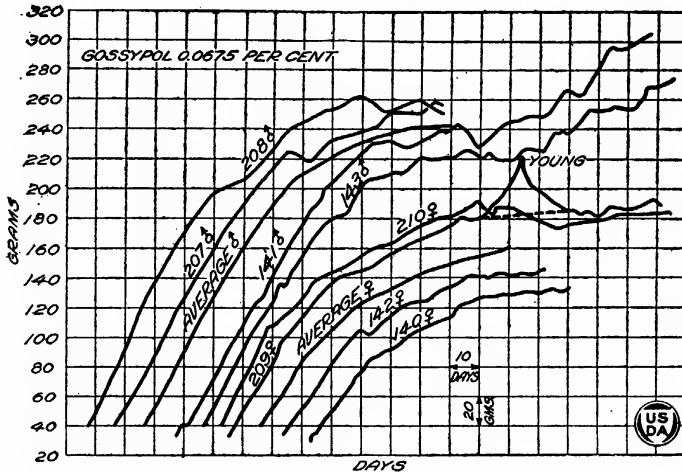


FIG. 6.—Effect of 0.0675 per cent gossypol in the diet. Broken line on one curve represents estimated weight had pregnancy not occurred

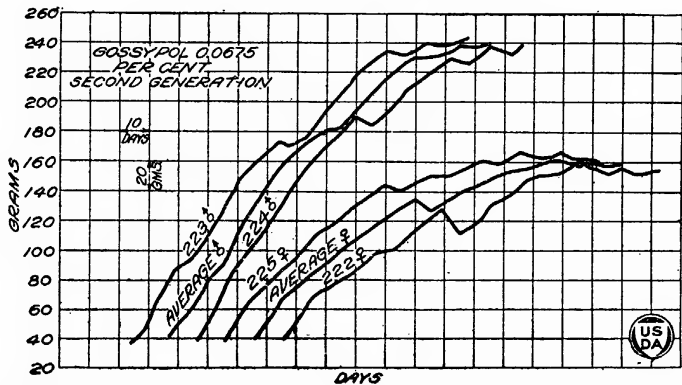


FIG. 7.—Effect of 0.0675 per cent gossypol in diet when fed to offspring of female rat raised on this diet and a male raised on control diet

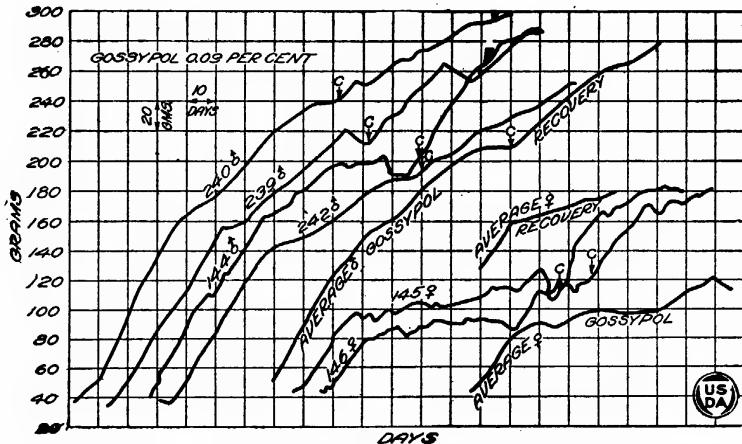


FIG. 8.—Effect of 0.09 per cent gossypol in the diet. “C” indicates change to control peanut meal diet. “Recovery” on “average” curves indicates period during which control diet was fed

the two remaining experiments is less marked. Two of the male rats when changed to the control peanut diet resumed growth immediately, although it was slower than in the case of the other two male rats. On the eightieth day the

four male rats averaged 207 gm., being 64 and 52 gm., respectively, below the average weight attained on the two control diets and 31 gm. below the average of the male rats fed on a diet containing 0.0675 per cent of gossypol. The average weight of the two female rats fed 0.09 per cent gossypol was below the average weight of the female rats on the other diets by 58, 38, and 35 gm., respectively.

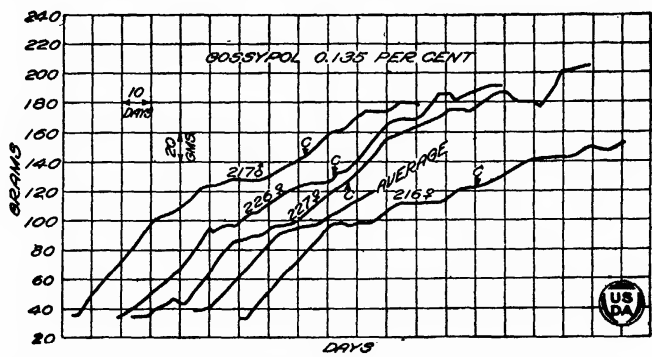


FIG. 9.—Effect of 0.135 per cent gossypol in the diet. “C” indicates change to control peanut meal diet

Only a few experiments were made with a concentration of 0.135 per cent of gossypol in the diet. The results, such as they are, indicate that the retardation of growth was marked and the resumption of growth poor. There is no question but that the poor resumption of growth is to be regarded as an effect of the gossypol, for the sturdiness of stock from which these rats came and the adequacy of the diet were certain.

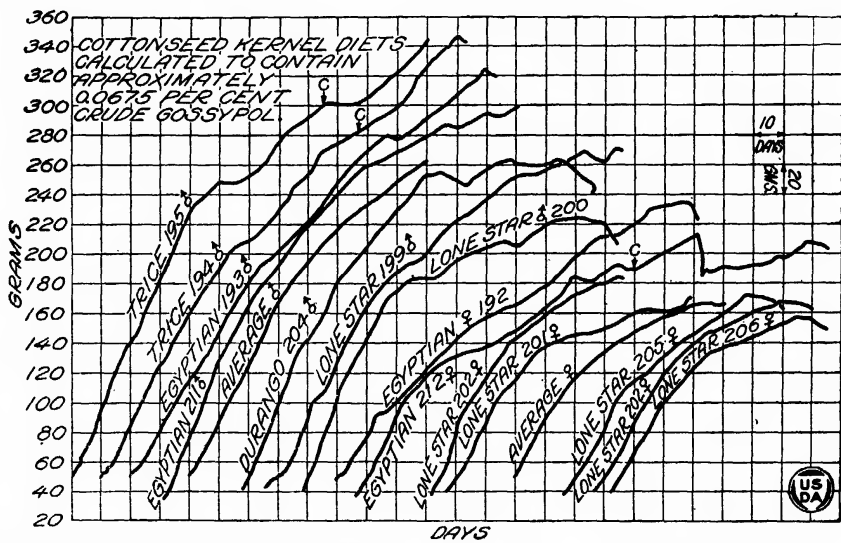


FIG. 10.—Effect of diet containing cottonseed kernels estimated to contain approximately 0.0675 per cent crude gossypol. “C” indicates change to control peanut meal diet

DIET CONTAINING ADDED RAW COTTONSEED KERNELS

The result of individual experiments in which raw cottonseed kernels were added to the diet in such proportions that the diet as a whole contained approximately 0.0675 and 0.135 per cent of gossypol are given in figures 10 and 11. Owing to the comparatively small number of experiments with each variety of kernel, separate average curves for each variety are not given. Instead, a composite curve for each sex for all kernels, based upon the average body weight of all the rats of each sex fed approximately the same estimated concentration of

gossypol, are presented. The rats fed cottonseed kernels grew on the average slightly better than the rats fed the estimated comparable quantities of gossypol.

In the case of the experiments with the cottonseed kernel diet estimated to contain 0.135 per cent of gossypol, the diet was withdrawn early and replaced by the control diet of peanut meal. The sudden change in the rate of growth is very marked, especially in the case of the males. On the sixtieth day the maximum difference between the body weight of the largest and smallest male rats was 70 gm. as compared with 30 and 35 gm. for normal rats on control diets.

It is also shown that the rate of growth on Lone Star seed was decidedly poorer than that with Egyptian and Durango seed. Seed of the last two varieties, however, were found by intraperitoneal injection of the ether extract to be as toxic as the analysis indicated, whereas the Lone Star seed was slightly less toxic than indicated. Such observations as these illustrate the necessity for a much larger number of experiments when exact estimates of toxicity are to be made in order to rule out experimental error due to variation in animals.

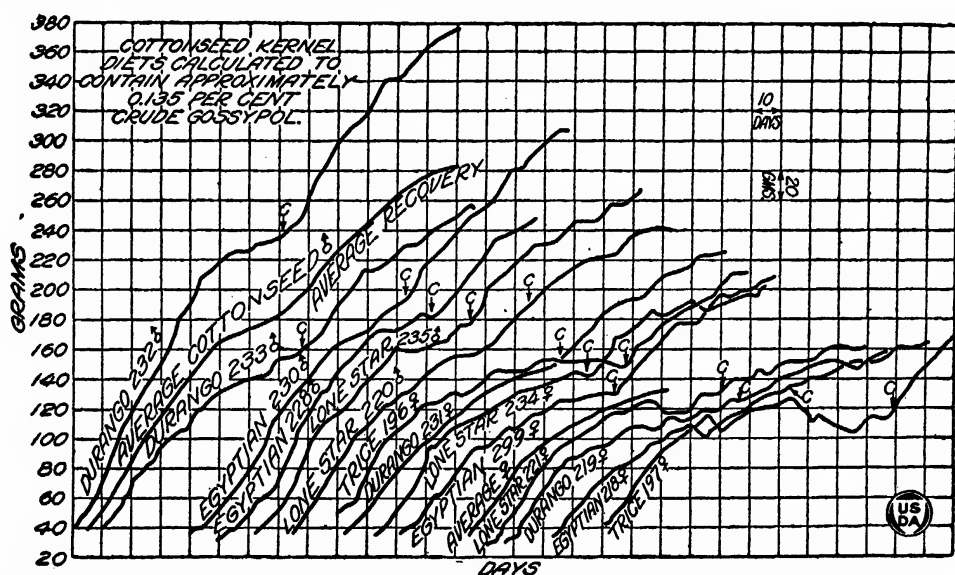


FIG 11.—Effect of diet containing cottonseed kernels estimated to contain approximately 0.135 per cent crude gossypol. "C" indicates change to control peanut meal diet. "Average recovery" indicates growth when all rats were receiving control diet

The cottonseed kernels fed in these experiments appeared to be slightly less toxic than was indicated by chemical analysis. The differences between the predicted and the observed toxicity, however, are not great.

Feeding tests with a diet containing 26.2 per cent of Durango cottonseed, estimated to contain 0.257 per cent of gossypol, are similar to those in which the diet contains 0.225 per cent of gossypol. Although in the Durango diet the estimated quantity of gossypol was a tenth greater than it was intended to be, this excess is within the limits of experimental error. The Durango seed, therefore, appears to be about as toxic as the gossypol analyses would indicate. The curves are given in figure 12.

The curves representing the rate of growth upon a diet containing 19.2 per cent of Egyptian cottonseed kernels, estimated to contain 0.227 per cent of gossypol, also presented in figure 12, are in practical agreement with the results of the tests in which the diet contained 0.225 per cent of added gossypol. The only difficulty in the way of stating that they are absolutely identical is the fact that one of the five rats on the Egyptian diet lived about twice as long as any of the



other rats. Since the significance of this single exception is not apparent, it may be concluded that for all practical purposes the Egyptian seed was as toxic as was indicated by the gossypol analysis.

The results of the feeding tests upon a diet containing 42.2 per cent of Lone Star cottonseed, estimated to contain 0.218 per cent of gossypol in the diet as a whole, are given in figure 12. They indicate that the actual toxicity is about equivalent to the toxicity of a diet containing 0.18 per cent of pure gossypol. By

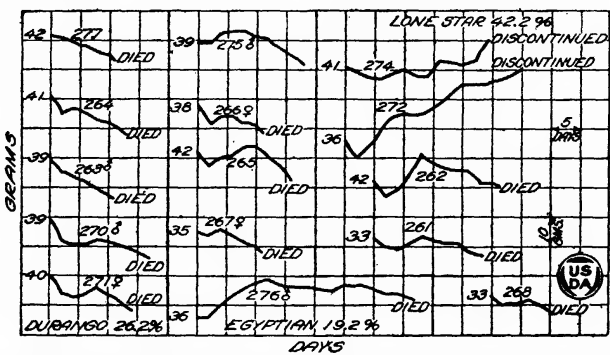


FIG. 12.—Effect of diet containing cottonseed kernels and estimated to contain approximately 0.225 per cent crude gossypol. Numbers to left of curve indicate the initial weight in grams and numbers above or below curve the laboratory number of animal. Because of short duration of experiments chart has been drawn on a scale twice that used in preceding chart

these tests, therefore, the Lone Star seed seems to be about 20 per cent less toxic than would be estimated from the gossypol analysis.

No experiments were made with a diet containing 54.6 per cent of Trice cottonseed kernels (estimated to contain 0.224 per cent of gossypol). In their place, experiments with 15 and 30 per cent of Trice kernels, the diets estimated to contain, respectively, 0.062 and 0.125 per cent of crude gossypol, are reported (fig. 13). These experiments, however, must be considered apart from those already re-



FIG. 13.—Effect of diet containing Trice cottonseed kernels estimated to contain approximately 0.0675 and 0.135 per cent crude gossypol, compared with a diet actually containing 0.0675 per cent gossypol. The eight experiments represented in this chart were conducted on rats from a single litter. They were performed during an interval when the laboratory was rather cool at night, and are therefore to be considered apart from others herein reported, in which the same diets were fed during warm weather or when the laboratory temperature regulator was in use. Because of the short duration of these experiments, the chart has been drawn on scale used in preceding chart

ported in which the same quantities of Trice cottonseed kernels were used during the warm weather. These experiments were performed on a single litter of rats at a time when the laboratory was cool at night, a condition which lowered the resistance of the animals. Compared with rats of the same litter which were fed a diet containing 0.0625 per cent of gossypol at the same time, the conclusion is that the Trice seed was probably from 67 to 75 per cent as toxic as was estimated from the gossypol analysis.

## REPRODUCTION, STUNTING, AND SENSITIVITY DUE TO AGE, TEMPERATURE, AND APPETITE

Grown rats, some of which had been raised on control diets, as well as some which had been raised on diets containing a small quantity of cottonseed kernels or gossypol, were fed the more toxic concentrations of cottonseed kernels or gossypol. The results of these tests vary somewhat and are not sufficient in number to warrant detailed consideration. Nevertheless, they show that the older rats were less sensitive than the younger ones. Perhaps this is due to the relatively small food requirements of the larger rats. In consequence, they would ingest less gossypol in proportion to their body weight than the smaller rats. Perhaps the loss of appetite, a symptom of gossypol intoxication, was a factor, for inanition is more serious for a young rat than for an old one.

Several litters of young were reared on a diet containing 0.0675 per cent of gossypol. There appeared, however, to be an undue proportion of breeding failures. With 0.135 per cent in the diet no litters were born, although breeding was attempted.

Post-mortem examination of several undersized rats revealed nothing abnormal except when a decided loss in body weight had occurred. The tail and body lengths and the weight of undersized rats which had not lost body fat agreed with those reported by Donaldson (4). The weights of the heart and liver were also within the normal limits for rats of their size. This touches upon a practical problem in nutrition as yet unsolved—the criterion by which stunting of a mild type and without evidence of malnutrition or disease is to be determined. Thus a possibility to be considered is that the feeding of certain quantities of cottonseed products containing gossypol under some conditions, while apparently compatible with perfect health, may produce undersized animals.

Temperature affects the resistance of animals to gossypol. In the winter and cool spring weather when the laboratory was not well heated at night young rats and grown mice succumbed to concentrations of gossypol which otherwise would have survived. This was also true of young rats fed diets containing raw cottonseed kernels. No mice were tried on this diet. These observations agree with the observation made on cottonseed meal, namely, that cold increases the sensitivity of the animals to it. In the authors' feeding experiments it did not appear that the cause of the increased toxicity was the increased consumption of food which caused an increase of gossypol intake per kilo of body weight of the animal. Presumably the deaths must be placed under the vague caption "exposure."

Gossypol and raw cottonseed kernels markedly affect the appetite, although the effect is not regular. It appears that one gets the normal weight variation plus a variation due to the effect of gossypol on the appetite. This is shown in some groups of animals in which the difference between the better and the poorer growths is greater in the case of animals fed gossypol or cottonseed than in those on the control diet.

The extent of growth followed the appetite or food intake. Comparisons, however, were made only when approximately the same concentrations of gossypol or comparable quantities of cottonseed kernels are involved and for the same sex. The food intake diagrams are not presented.

## DISCUSSION

The experiments in which pure gossypol dissolved in peanut oil was injected intraperitoneally and those in which the ether extract of different varieties of cottonseed was injected intraperitoneally show a general similarity in toxicity. The Egyptian and Durango seeds showed the toxicity that was to be expected from their gossypol content. The Lone Star and the Trice seeds, however, deviated somewhat from the expected toxicity. Chemical analysis of the seeds studied showed an extreme range in gossypol content of 300 per cent, whereas the range in toxicity of the ether extract from these seeds was from 400 to 450 per cent. This was due, not to a toxicity of the seeds in gossypol greater than that corresponding to their gossypol content, but rather to a lower toxicity of the seed relatively low in gossypol. It is to be expected, of course, that where the gossypol content is low, the errors of the analytical method will be exaggerated as compared with those seeds in which the gossypol content is high. Furthermore, it is to be expected that decomposition and modification of the gossypol will be relatively greater in the seeds with a low gossypol content than with those having a high gossypol content. These are the main factors in accounting for the differences between the calculated and the observed toxicity of different quantities of seed. The conclusion that the experiments with the intraperitoneal injection of gossypol itself and of ether extract of cottonseed show that the two are equally toxic and that gossypol is the only or at any rate the predominant toxic principle of cottonseed, seems to be warranted.

The feeding experiments with diets containing either added pure gossypol or raw cottonseed kernels added to the diet in such proportion that the gossypol content of the total diet was known show the same parallelism between gossypol content and toxicity. For diets containing quantities of gossypol large enough to cause death or arrest growth in a relatively short time this is entirely clear. With smaller quantities in the diet such parallelism, of course, is much less clear and is not to be expected. The symptoms observed from feeding gossypol and cottonseed kernels were similar. They consisted of loss of appetite and depression of growth, which, if sufficient quantities of gossypol or of cottonseed kernel had been fed, often led to death, if the feeding was continued. If the feeding was discontinued and the animals changed to control diet, growth was resumed at varying rates. There was some evidence that animals were permanently injured when fed for long periods on the gossypol or raw cottonseed kernel diets.

The results herein recorded could not have been due to any defect of the diet as such. A superabundance of protein, of vitamin, of salt, etc., was furnished so as to give the best opportunity for growth and to diminish as much as possible any indirect effect of the poison. Moreover, when additions were made to the control diet, care was taken to keep the protein content of the diet practically the same. It is obvious that merely to have added cottonseed products to the control diet would have changed its character, particularly with respect to protein, and would have introduced a further variable into the experiment.

Perhaps the results obtained in this investigation explain the discrepancies between the experiments of Richardson and Green (8) and those of Osborne and Mendel (6) with 9 per cent of protein (in the form of cottonseed flour) in the diet. Different degrees of growth were obtained. The reason for this may lie in the gossypol content of the different samples of cottonseed flour used by these investigators. At that time it was impossible to estimate the gossypol content, for no methods had been developed.

It is evident that henceforth a knowledge of the gossypol content is essential in any investigation of the toxicity and nutritional value of cottonseed press cake. Moreover, the data presented in this paper do not permit generalizations concern-

ing the toxicity of cottonseed press cake. Its toxicity will vary greatly with the variety of seed and with the method of preparation and subsequent treatment of the press cake. Moreover, the rat is a relatively resistant animal. In the experiments herein reported, as well as in some of the experiments reported in the literature, conditions very favorable to the well-being of the animal were usually provided. Before generalizations concerning the toxicity of cottonseed products can be made, further work must be done upon other species of animals and upon animals kept under different conditions.

### SUMMARY

A comparison of the toxicity of pure gossypol with that of four varieties of cottonseed kernels was made. The cottonseed kernels contained known quantities of gossypol. Pure gossypol in peanut oil solution and the ether extract of cottonseed kernels were injected intraperitoneally into rats. The toxicity of the cottonseed kernel extracts corresponded to their gossypol content with moderate variations. Rats were fed upon a totally adequate diet to which were added in some cases known quantities of gossypol and in other cases raw cottonseed kernels in which the gossypol content had been determined. The toxicity of these diets with moderate variations corresponded to their gossypol content.

The results herein reported bear out the conclusion that the toxicity of cottonseed kernels is due to their gossypol content. Inasmuch as the gossypol content of cottonseed kernels varies in the different producing regions, the observed regional variation in the toxicity of raw cottonseed kernels is explained.

Accordingly, this tendency to geographic variation in the toxicity of the raw material would be expected to affect the toxicity of cottonseed press cake and meal made in different regions by the same manufacturing process. Confirmation of this conclusion is found in the fact that in the Atlantic Coast States, where the gossypol content of cottonseed averages slightly less than 1 per cent, cottonseed poisoning is more feared than in the Southwest, where gossypol occurs in cottonseed in smaller quantities.

In this investigation no attempt has been made to study the variations in toxicity which might be due to different methods of treating the seed in the manufacturing process. No attempt was made to study the special procedures of individual investigators in this field. The effort has been limited to establishing the toxicity of the raw material used in the manufacture of the meal. Differences in this respect are at present beyond control. They must be taken into account both in research upon this subject and in the practical use of the material in industry and agriculture. Finally, an experimental basis has been found which makes it possible to correlate the divergent views and the apparently contradictory findings of various investigators.

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# PHARMACOLOGY OF GOSSYPOL<sup>1</sup>

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## INTRODUCTION

The gossypol content of cottonseed kernels varies in the different cotton-producing regions of the United States (9)<sup>2</sup> and the toxicity of different lots of kernels is directly proportional to their gossypol content (10). The rats used as test animals for the experiments on which these conclusions were based, however, did not exhibit all the manifestations of the cottonseed poisoning of farm animals, namely, diarrhea, loss of appetite, loss of weight, shortness of breath, and paralysis. Edema of the anogenital region also sometimes occurs and post-mortem examination may reveal effusion into the serous cavities, edema of the lungs, hypertrophy of the heart, and neuritis. Rommel and Vedder (8) have pointed out the resemblance of this symptom complex to beriberi. Of all these effects of cottonseed poisoning rats showed only loss of appetite and loss of weight. To remove any doubt as to the identity of the agent causing these intoxications of rats and of the causative agent of cottonseed poisoning of farm animals, experiments upon animals other than rats were undertaken.

## ANIMALS USED

As the Bureau of Chemistry has no facilities for experiments on farm animals, the work was done on cats which are carnivorous and are particularly well suited to experiments in which inanition may become a complicating factor because they are less likely to succumb to acidosis than herbivorous or omnivorous animals. The results of this investigation upon the toxicity of gossypol for cats, together with some additional observations on rabbits, guinea pigs, rats, and mice, are here presented.

## RATIONS FED

In the rabbit feeding experiments the gossypol was added in ether solution to the oats of the diet, the ether being removed subsequently by evaporation. A small ration of carrots or cabbage was also fed daily. Since this treatment of oat kernels presumably causes the gossypol to be deposited on the oat hulls, rabbits which hull their oats were rejected. In the cat feeding experiments, meat to which gossypol dissolved in butterfat had been added was refused. The solution of gossypol in butterfat was therefore mixed with skim milk powder and this mixture was incorporated in the hashed lean meat upon which the cats were fed. Meat is moist and does not necessarily take up gossypol as would dry material and it is impossible to work up the hashed meat and the milk powder mixture into a homogeneous mass. This method of administering gossypol therefore is not ideal when the effect of gossypol upon digestion is being studied. In all of the feeding experiments, except those performed to determine the effect of different concentrations, the dosage was so adjusted as to feed the maximum quantity of gossypol compatible with the maintenance of a fair appetite.

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<sup>1</sup> Received for publication Mar. 21, 1924.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 197.

## EFFECT OF GOSSYPOL

One of the effects of feeding gossypol was diarrhea. Another was loss of appetite, which usually occurred soon after the administration of as little as 450 parts per million in the diet to both cats and rabbits, although some rabbits withstood this concentration for a time. The highest concentration on which the appetite of rabbits was maintained was 225 parts per million of gossypol in the diet. No disturbances of appetite in cats were noted from 112 parts of gossypol per million of diet, but with 225 parts loss of appetite was common. In comparing the two species allowance must be made for the high moisture content of the cat diet as well as for the absolute bulk of food eaten. Rats apparently are decidedly less sensitive, for they withstand from three to six times greater concentrations of gossypol in the diet, despite the fact that in proportion to their size they consume larger quantities of food than cats or rabbits (10).

## METABOLISM OF ANIMALS UNDERGOING GOSSYPOL POISONING

A study with gossypol similar to that of Wells and Ewing (11) on the metabolism of animals undergoing cottonseed poisoning seemed desirable. Especially did it seem important to maintain the appetite of the test animals in order to avoid the nitrogen fluctuation which in the experiments of Wells and Ewing may have obscured one of the effects of the intoxication. While some trouble was encountered in keeping the animals eating the entire ration, this was done successfully in the case of one cat, although after several feedings of gossypol, the ration was not consumed as rapidly as before. There was also some trouble from diarrhea. This was controlled by feeding bone ash and also by cutting the food allowance to the minimum.

The data on the intake and outgo of nitrogen are given in Table I. During the laboratory hours the urine was collected when voided, and preserved. At the end of the periods, which lasted from one to several days, the urine was always pressed out of the bladder and caught in a graduated cylinder. Only gentle house-broken female cats were used. The reliability of this procedure for dividing the experiment fairly accurately into periods of 24 hours or multiples thereof was also demonstrated by data on control periods. Many times no urine was voided during the 24 hours. Complete specimens were therefore pressed out into the cylinder at one time. A charcoal marker was used so that the feces could be separated. Usually periods longer than those used in collecting the urine were employed.

It seems justifiable to conclude that for the period during which the diet consisted of a daily ration of ground lean meat, prepared at the beginning of the experiment, the administration of gossypol caused a negative nitrogen balance, the animal in the fore-period having been practically in nitrogen equilibrium. This conclusion is in harmony with the results of Macy and Mendel (5). The animals to which they fed cottonseed or cottonseed meal generally lost more weight than the control animals which in each of their experiments received exactly the same quantity of food as that eaten the previous day by the test animals.

The interpretation of the data on the fecal nitrogen is less clear, owing to a lack of certainty of precise separation of feces by markers, and the slightness of the increase of fecal nitrogen when gossypol was fed. Moreover, the markers may have had some effect on the fecal nitrogen. It seems probable, however, that the administration of gossypol caused a somewhat increased elimination of nitrogen through the feces. It is less than is to be expected from the results of Jones and Waterman (4), Crowther and Woodman (3), Mendel and Fine (6), and Rather (7). Jones and Waterman, using gossypol prepared by the writers, found that it retarded the digestion of cottonseed globulin and of casein *in vitro*.



TABLE I.—Metabolism of cat No. 30 undergoing gossypol poisoning

DIET: 100 GM. MEAT, 2 GM. BUTTERFAT, 2 GM. SKIM MILK POWDER

Date	Weight	Bone ash fed	Nitrogen daily				Gossypol administered
			Fed	Urine <sup>a</sup>	Feces	Balance	
1920	Grams	Grams	Grams	Grams	Gram	Gram	
Oct. 26-31	{ 1,820 1,855 1,890 }	{ 2 2 2 }	3.42		0.077		Fore-period.
Nov. 1-4	{ 1,985 1,890 1,825 }	{ 2 2 4 }	3.42		.312		90 mgm. daily.
5	1,890	2	<sup>b</sup> 2.01		.055		
6-7 <sup>c</sup>	{ 1,795 1,825 }	{ 4 4 }	3.33		.192		None.
8	1,825	4	3.33	2.641	.192	+0.497	
9	1,830	4	3.33	2.697	.143	+ .490	
10	1,820	4	3.33	2.725	.143	+ .462	
11 <sup>c</sup>	1,825	4	3.33	2.599	.206	+ .525	
12	1,880	4	3.33	2.599	.206	+ .525	45 mgm. daily.
13 <sup>c</sup>	1,865	4	3.33	3.117	.206	+ .007	
14 <sup>c</sup>		4	3.33	3.117	.206	+ .007	
15	1,855	4	3.33	3.302	Lost.		
16	1,865	4	3.33	3.302	do		
17	1,850	4	3.33	3.397	do		
18	1,860	4	3.33	3.397	do		

DIET: 75 GM. MEAT, 2 GM. BUTTERFAT, 2 GM. SKIM MILK POWDER

Dec. 11	1,843	2	2.617	2.640	0.061	-0.084	Fore-period.
12		2	2.617	2.640	.061	-.084	
13	1,835	2	2.617	2.640	.061	-.084	
14		2	2.617	2.515	.061	+ .041	
15	1,840	2	2.617	2.515	.061	+ .041	
16	1,840	2	2.617	2.690	.182	-.255	45 mgm. in butterfat and milk powder daily.
17 <sup>c</sup>	1,875	2	2.617	2.599	.182	-.164	
18		2	2.617	2.802	.182	-.367	
19		2	2.617	2.802	.182	-.490	
20 <sup>c</sup>	1,840	2	2.617	3.068	.245	-.696	
21	1,850	2	2.617	3.068	.245	-.696	
22	1,820	2	2.617	3.068	.245	-.696	
23 <sup>c</sup>	1,780	2	2.617	3.068	.245	-.696	
24	1,780	2	2.617	2.456	.1414	+.020	After-period.
1921							
Jan. 4	1,765						

<sup>a</sup> No albuminuria.  
<sup>b</sup> Net quantity fed after animal vomited.  
<sup>c</sup> 1 gm. of charcoal fed.

TABLE II.—Toxicity of gossypol

Animals used	Injected intraperitoneally in peanut oil			Animals used	Injected intravenously as sodium salt		
	Usually survived	Average lethal	Certain lethal		Usually survived	Average lethal	Certain lethal
	Mgm. per kgm.	Mgm. per kgm.	Mgm. per kgm.		Mgm. per kgm.	Mgm. per kgm.	Mgm. per kgm.
5 rabbits	5	10	20	10 rabbits		35-40	50-
2 cats	40-80			9 cats	50		75
20 rats	20	30-35	50	8 rats	35	40-50	50+

The other investigators reported a low digestibility of the protein in cottonseed as measured by fecal nitrogen. The smallness of the increase found in the present investigation may be due to the manner of administering the gossypol. Perhaps it did not come in close enough contact with the meat protein of the cat's food. Moreover, the experiments herein reported were conducted so that absorption was favored by cutting the diet down to a low level and by checking the tendency to diarrhea with bone ash. In an experiment conducted without bone ash and with a large dose of gossypol the diarrhea was marked and the fecal nitrogen was high.

### PARALYSIS FROM GOSSYPOL

When the gossypol content of the rabbits' diet was adjusted to between 225 and 450 parts per million, two of the rabbits which retained a fairly good appetite developed spastic paralysis of the hind legs within two months. The hind legs were stiff and protruded forward upon the abdomen (similar to cat shown in Pl. 2, B). The anogenital region became edematous and ulcerated and there was dribbling of urine. Paralysis also occurs in cats (Pl. 1 and 2), the hind legs being more often affected. In several cases in which only the fore legs seemed to be affected there was reason to suspect that the hind legs were also involved, although not enough to be apparent. The paralytic condition may become progressively worse for a time after the gossypol diet is discontinued or may improve while the gossypol diet is being continued. The kangaroo hop (Pl. 2, C and D) illustrates the effort of the animal to compensate for the paretic condition. Even the tail is brought into play as an accessory organ of locomotion.

Paralysis was observed not only in rabbits continuously fed gossypol but also in one rabbit which survived intraperitoneal injection of 5 mgm. of gossypol per kilo dissolved in peanut oil, and in one receiving orally 50 mgm. of gossypol per kilo dissolved in peanut oil. Only these two individuals out of a large number of rabbits which were injected showed paralysis. The paralysis developed within 10 to 20 days, being preceded only by weakness. Within several more days the hind legs became spastic and extended. The hind legs were affected unequally. The inequality was especially well shown in the behavior of the tail, which turned to one side. Almost complete recovery occurred within two months.

Although on injecting other animals the paralysis could not be reproduced, it is believed that the paralysis of the two rabbits described was due to gossypol, for among the several thousand rabbits observed in the pharmacological laboratory of this Bureau spontaneous paralysis has never been encountered. Moreover, the paralysis resembled exactly that found on feeding gossypol. It is believed that these observations on the feeding or injection of gossypol are the first recorded instances of a paralysis in rabbits which is attributable to gossypol or to cottonseed products.

Gossypol has a paralytic effect not merely upon the neuromuscular apparatus of striated muscle but also upon that of smooth muscle. At any rate it produces definite paralysis of the isolated rabbit intestines in concentration of about 1 to 5,000 within about 15 minutes. With more dilute solutions, such as 1 to 100,000, the effect was not so certain. Post-mortem examination of rats dead of intraperitoneal injection of gossypol frequently reveals a condition resembling paralytic ileus. As cottonroot bark which contains gossypol is regarded as an abortifacient, some effect upon the smooth muscle of the uterus might be anticipated. However, while no experiments were made upon the isolated uterus, abortion could not be effected in pregnant cats or rabbits by poisoning them with gossypol. The few fetuses which were born succumbed and presumably would have been born anyway. In one case only part of the litter was born. When pregnant animals poisoned by gossypol were sacrificed, live fetuses were found and no separation of placentae was observed.

In the continued feeding experiments with rabbits evidence of the action of gossypol on the neuromuscular apparatus is found in the dribbling of the urine. This, however, was associated with motor paralysis, so that the point of attack might have been in the cord.

### EFFECT OF GOSSYPOL ON CIRCULATION

Gossypol apparently also affects the neuromuscular apparatus of the heart, for frogs that have died from the slow absorption of gossypol injected in oil solution into the ventral lymph sac show a peculiar spotted condition of the ventricle which presumably is associated with the fact that portions are in systole or rigor while other portions are fully stretched. In higher animals death comes from cardiac failure. Primarily the respiration is not at all or only slightly affected.

Gossypol is a circulatory depressant. It produces a fall in blood pressure, usually immediately after it is injected. A recovery of pressure may occur gradually. Section of the vagi may bring it back to normal immediately, or, especially in rabbits, to above normal. For some time after injections of gossypol the heart shows skipped or weak beats. Once a two to one block was obtained which later disappeared spontaneously. Nevertheless, two cats receiving gossypol in continuous feeding experiments which died under observation gave no clinical evidence of irregularity of the heart rhythm. One cat, a good feeder, which received gossypol continuously and which died with edema of the lung, had decided shortness of breath and rapid pulse and breathing before death. The enlarged heart is shown in Plate 2, a. Several other cats showed the same symptoms.

### EDEMA FROM GOSSYPOL

Edema may follow gossypol injections as well as gossypol feeding. Marked edema of the ears of rabbits develops when gossypol solutions are injected into the ear veins, presumably owing to a slight leakage of the solution into the perivascular tissue. Edema also occurs around oil solutions of the gossypol in the subcutaneous tissue. Marked serous exudation follows in cats, rabbits, and rats when peanut oil solutions are injected intraperitoneally. In several cats which survived and subsequently were examined the ascites was intense and protracted. The injection of the oil free from gossypol does not ordinarily give rise to any visible serous exudate in the case of rats and only very little in the case of cats or rabbits.

When gossypol is injected intravenously in alkaline solution, or intraperitoneally in oil solution, edema of the lungs occurs in rabbits, guinea pigs, cats, rats, and mice. This is more intense and appears sooner the larger the dose. The fatal edema is hemorrhagic and red exudation may appear at the nose. Edema of the lungs also occurs in cats in chronic intoxication from gossypol feeding. Some effusion into the serous cavities may be noted. Measurements were made of the limbs of paralyzed cats but no disproportion was evident. Upon recovery from paralysis there was possibly a slight decrease in the size of the limbs, perhaps connected with the disappearance of an edema.

### POST-MORTEM EXAMINATION

Data on the toxicity of gossypol administered intravenously in fixed oil are given in Table II. The rabbits which died did so usually within one to two days, whereas the cats, except those given large doses, lingered for four to five days. The time of death of the rats varied.

Examination by the Marchi method of the sciatic nerve from several paralyzed cats revealed degeneration. Dr. M. C. Winternitz, who also examined the experimental animals of Macy and Mendel (5), very kindly made histological

examinations of the lungs of four rabbits dying from the intravenous injection of 50 mgm. of gossypol per kilo in filtered alkaline solution. He reported in part as follows:

I find in three of the animals (rabbits Nos. 114, 118, and 119) a congestion of the alveolar capillaries, fairly diffuse edema of the lung, and in several sections extravasations of red blood cells into the albuminous exudate within the alveoli. On the other hand, rabbit No. 117 shows a relatively normal lung tissue. In one animal (rabbit No. 114) polymorphonuclear leukocytes and fibrin also occur in small foci. The pneumonic areas in this rabbit are the more interesting because there is no exudate in the lumen of the bronchi. In rabbit No. 118 there is a relatively slight admixture of either red or white cells and fibrin in the exudate. In rabbit No. 119 there is no outspoken purulent pneumonic condition.

The frequent lesions observed in Macy and Mendel's series of subacute and chronic intoxication were congestion and hemorrhage in the intestines, congestion of the liver and kidneys, and edema of the lungs. The lungs examined by Doctor Winternitz for the writers came from animals that had succumbed to acute intoxication. One animal died before pulmonary complications had time to develop. Three animals showed fairly diffuse edema, congestion of alveolar capillaries, and, in a few places, extravasation into the exudate in the lumen of the alveoli. The pneumonic condition was secondary. Marked cardiac enlargement (Pl. 2, a) was observed in the cat described as having had dyspnoea before death. The other cats with similar symptoms did not give the positive evidence of cardiac enlargement that was shown by this animal.

#### SUMMARY

Gossypol has been fed for long periods to cats and rabbits in dosages small enough to avoid marked loss of appetite, yet large enough to produce chronic intoxication. The symptoms and lesions observed were loss of appetite with the larger dosages, paralysis, with nerve degeneration, shortness of breath, cardiac hypertrophy, edema of the anogenital region and of the lungs, and effusion into the serous cavities. Spontaneous recovery may occur temporarily while gossypol is being administered and the condition of the animal may become progressively worse for some time after it is discontinued.

Following the subcutaneous, intravenous, and intraperitoneal injection of gossypol, there is local edema of the lungs, which may be hemorrhagic, and effusion into the serous cavities. Blood pressure falls, heart action becomes irregular, and death comes from cardiac involvements.

Pure gossypol is capable of producing nearly all the manifestations reported as characteristic of the cottonseed intoxication of farm animals. Therefore the conclusion seems warranted that gossypol is the principal causative agent of cottonseed poisoning. This conclusion is supported by the fact, established in earlier papers of this series (9, 10), that the toxicity of raw cottonseed kernels varies nearly directly with their gossypol content, or is only slightly less. Thus there can not be much of any other toxic substance in cottonseed kernels unless it be some decomposition product of gossypol. The pharmacology of such products has not been investigated.

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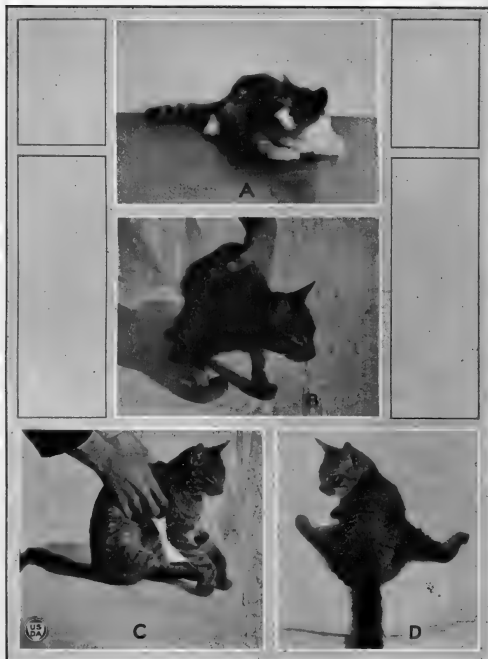
## PLATE 1

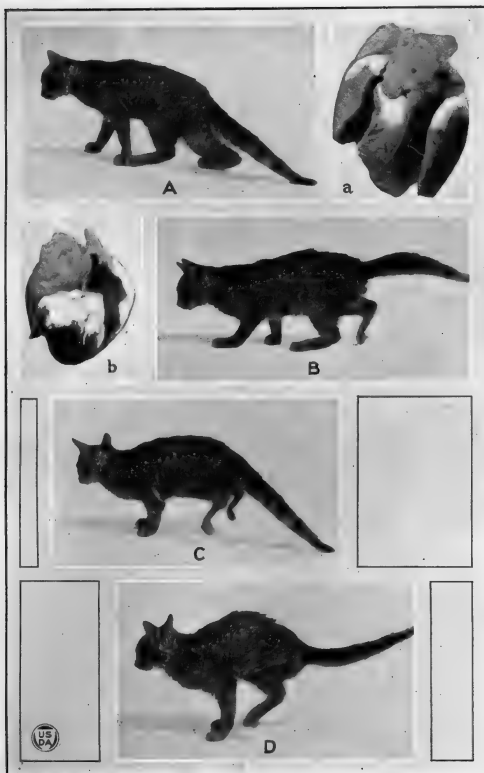
A.—Early stage of paralysis of fore legs, with the inward curl. The legs become crossed when the animal is raised.

B.—Unequal paralysis of fore legs. This position was often assumed as the cat walked.

C.—Double-jointed effect often seen as the cat walked.

D.—Spastic paralysis of unequal degree in hind legs of cat No. 11 (September 22, 1920). The tail was completely extended. Locomotion was accomplished by the fore legs, the buttocks and abdomen being dragged on the floor. Rabbits also show this type of paralysis. In earlier stages, paralysis was of a more flaccid type.







## PLATE 2

A and B.—Partial recovery of cat No. 11 (October 14, 1920) from stage shown in Plate 1. It has a slight weakness of the fore legs, a greater weakness of the hind legs, and the quick step of the multiple neuritis type of paralysis. (Negative exposed for one-fiftieth of a second.)

C and D.—Unusual form of rapid locomotion shown by cat No. 11 (October 14, 1920). The back is arched and the tail is used as an aid in locomotion. (Negative exposed for one-fiftieth of a second.)

a and b.—(a) Enlarged hypertrophied and dilated heart of cat No. 11, and (b) heart of cat No. 19 (control animal). The large heart (a) weighed 12.35 gm. The net body weight of cat No. 11, which was about 32 weeks old, was 1,475 gm., exclusive of the serous exudate (215 gm.). Its previous maximum body weight was 1,890 gm. The body weight of cat No. 19, about 29 weeks old, was 2,405 gm. and the weight of its heart was 6.1 gm. The weight of the enlarged heart was twice that of the heart of a normal cat weighing 1,465 gm. The enlarged heart has much less epicardial fat than the smaller one.

# JOURNAL OF AGRICULTURAL RESEARCH

## CONTENTS

	Page
The Relation of Antecedent Egg Production to the Sex Ratio of the Domestic Fowl M. A. JULL	199
A Gradient of Permeability to Iodin in Wheat Seed Coats HARRY BRAUN	225
Further Studies in the Relative Susceptibility to Citrus Canker of Different Species and Hybrids of the Genus Citrus, Including the Wild Relatives GEORGE L. PELTIER and WILLIAM J. FREDERICH	227
Relation of Environmental Factors to Citrus Scab Caused by <i>Cladosporium citri</i> Massee GEORGE L. PELTIER and WILLIAM J. FREDERICH	241
A Study of Ensiling a Mixture of Sudan Grass with a Legume P. A. WRIGHT and R. H. SHAW	255
Bacterial Leafspot of Delphinium MARY K. BRYAN	261
Further Observations on the Molts of the Ox Bots <i>Hypoderma bovis</i> De Geer and <i>H. lineatum</i> Villers E. W. LAAKE	271
<i>Flavobacterium suaveolens</i> , a New Species of Aromatic Bacillus Isolated from Dairy Wastes LULU SOPPELAND	275
Some Relations between the Growth and Composition of Young Orange Trees and the Concentration of the Nutrient Solution Employed H. S. REED and A. R. C. HAAS	277
The Germination of Cottonseed EBEN H. TOOLE and PEARL L. DRUMMOND	285
The Salt Content of Cotton Fiber THOMAS H. KEARNEY and C. S. SCOFIELD	293

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No. 3

## THE RELATION OF ANTECEDENT EGG PRODUCTION TO THE SEX RATIO OF THE DOMESTIC FOWL<sup>1</sup>

By M. A. JULL

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### INTRODUCTION

In the domestic fowl comparatively little work has been done to determine the normal sex ratio and still less has been done to determine what factors might operate toward a possible modification of the normal sex ratio. As a contribution in this direction an experimental study was undertaken to determine the sex ratio in the domestic fowl from the time of commencing to lay to practically the end of the first year of production. Under the direction of Dr. L. J. Cole, of the department of genetics of the University of Wisconsin, the plan of the work was originally outlined in 1919 and was continued for three consecutive years. Among other things the plan of work involved a study of the following topics:

1. The normal sex ratio.
2. The relation of prenatal mortality to the sex ratio.
3. The relation of egg production to the sex ratio.
4. The relation of egg weight to the sex ratio.
5. The relation of yolk weight to the sex ratio.
6. The relation of egg water content to the sex ratio.

In this paper it should be understood that the term "sex determination" has reference to the causes which lead to the production of an individual of one or the other sex. During recent years considerable progress has been made in arriving at a more complete understanding of the causes which determine sex but this does not necessarily imply an increased ability to control sex. The term "sex control" is understood to imply that the causes which determine sex are more or less amenable to human control.

There are many investigators who believe that the presence or absence of a second sex chromosome constitutes one link only in a series of events that precede the development of sex. The opinions of these investigators range from those in which the sex chromosomes are regarded as playing an insignificant rôle in sex determination to those in which the internal mechanism is regarded as being really responsible for the control of sex but where external conditions may be operative to a minor extent in modifying sex development. The question naturally arises as to whether sex determination involves an irreversible tendency to the corresponding sex differentiation, or whether such differentiation may be controlled or even reversed.

<sup>1</sup> Received for publication April 26, 1924. The experimental part of the work was conducted in the department of genetics, Agricultural Experiment Station, University of Wisconsin, and at Macdonald College, St. Anne d'Bellevue, P. Q., Canada. The study was completed in the U. S. Department of Agriculture. Published with the approval of the director of the Wisconsin station as Paper No. 46.

## EXPERIMENTAL STUDY OF THE SEX RATIO IN THE DOMESTIC FOWL

## MATERIALS

During the first year of this experimental study reciprocal matings were made between Barred Plymouth Rocks and single-comb White Leghorns. Two pens of birds were used, each pen consisting of five Barred Plymouth Rock and five White Leghorn pullets, the first pen being mated to a Barred Plymouth Rock cockerel and the second to a White Leghorn cockerel. In the matings between Barred Plymouth Rocks and White Leghorns all the chicks were either entirely or largely white, the white of the White Leghorn being dominant to the sex-linked barring factor of the Barred Plymouth Rock.

During the last two years matings were made between Barred Plymouth Rock pullets and single-comb Brown Leghorn cockerels. The mating for the year 1920-21 consisted of 10 pullets and 1 cockerel and for the year 1921-22 two pens were mated, each consisting of 10 pullets and 1 cockerel. In these matings the sex-linked barring factor of the Barred Plymouth Rock is dominant to the Brown Leghorn plumage coloration. The male progeny is always barred, with shanks of the normal yellow color of both parental breeds. The females are always black or black tinged with brown, with black-pigmented shanks. At hatching time it is very easy to distinguish the sexes, since the males invariably have a small patch of white down on the back of the head and the shanks are always light in color, while the females have no white patch on the back of the head and the shanks are always black or very dark in color. The identification of the sex at hatching time is quite reliable, and during the two years out of 1,370 chicks only six errors were made in identifying the sex prior to dissection or observation of the secondary sexual characters of the growing birds.

As far as the observations in this study are concerned, it can be said that external appearances of the chicks at hatching time give no indication of reversibility of sex. The characteristic secondary sexual features of the male were always associated with a male as shown by dissection, and so with all females. Birds which were allowed to reach the adult stage showed no signs of an intermediate sex condition or of changing from one sex to another.

Of the 50 females used in this study, the records of only 45 are analyzed, since either through early death or very poor production the records of 2 birds for each of the first two years have been discarded and in the third year female No. 1508 produced not 1 fertile egg out of 63 laid.<sup>2</sup>

## METHODS

All of the birds were trap-nested throughout each year. Complete records were kept of production, and during the last two years the eggs were weighed daily. Throughout the three years the eggs were placed in the incubators on an average of about every 10 days. From the time of gathering the eggs to the conclusion of the work each season practically every egg has been accounted for. All cracked eggs have been recorded, whether they were cracked by the hen in the nest, by the attendant in gathering the eggs, or by the operator during the period of incubation. Detailed records were kept of all eggs which were infertile, embryos which died before the twelfth day of incubation and on which sex could not readily be obtained, embryos which died on and after the twelfth day

<sup>2</sup> The first and last year's work was done in the department of poultry husbandry of Macdonald College, while the second year's work was done in the department of genetics of the University of Wisconsin, the birds being very kindly supplied by Prof. J. G. Halpin, of the department of poultry husbandry of that institution.

of incubation and on most of which the sex was determined. During the normal hatching season the chicks which hatched were banded, and a note was made of the sex, which was later checked by observation of the secondary sexual characters. Up to and including the time of the normal hatching season, the chicks were allowed to hatch, and whatever chicks hatched were killed and dissected immediately. Subsequent to the hatching season the eggs were removed from the incubator about the nineteenth day and were all dissected. Occasionally an egg would become lost, usually through being broken in attending the incubator and no record being made. The sex on a few chicks was undetermined, either through two or more chicks getting together in the hatching trays or through chicks becoming lost during the growing season. Although, under normal conditions, the sources of loss of records among growing chicks are numerous, including loss from enemies of the poultry flock and loss of leg-band before the insertion of the permanent wing band, the number of eggs and chicks in this experiment on which no records were obtained was small indeed. Furthermore, it is probably true that all cracked and missing eggs and all missing chicks would constitute a random sample of the entire population as far as the matter of sex ratio is concerned.

The weights of the eggs are considered in relation to production and to sex ratio. Using another group of birds, the weights of the yolks of all eggs laid during the first year of production have been determined and are considered in relation to production and to sex ratio. The water content of a number of eggs laid at different periods of production has been determined, and this also is considered in relation to production and to sex ratio.

### RESULTS

Before undertaking the presentation of the results from this experimental study, it is desirable to call attention to one or two matters. The sex ratio is presented in terms of the percentage of the males in the total population. In subsequent sections of this paper the sex ratio will be given in terms of the male ratio and will be designated as  $R\delta$ .

In the context which follows the following symbols have the attached significance:  $R\delta H$  = per cent of males in all chicks which hatched;  $R\delta D$  = per cent of males among all dead embryos in which sex was determined.

TABLE I.—*Summary table showing complete results of the three-year period 1919-22*

Year	Number of birds	Total egg production	Cracked eggs	Infertile eggs	Dead embryos not sexed	Missing eggs and chicks	Total females	Total males	Total sexed	Sex ratio
1919-20.....	18	1,845	116	132	564	7	550	476	1,026	46.39±0.64
1920-21.....	8	979	57	217	199	5	246	255	501	50.89±0.85
1921-22.....	19	1,779	82	551	251	26	440	429	869	49.37±0.66
Total.....	45	4,603	255	900	1,014	38	1,236	1,160	2,396	48.41±0.47
Per cent.....			5.54	19.55	22.03	0.82	26.85	25.20	52.05	

Table I gives the mean sex ratio of the experimental fowls for the whole three years. It will be noticed that the sex ratio for the second year is higher than for either of the other two years, but it should be explained that during the second year two of the eight birds gave very high male ratios throughout the year.

The question which arises is whether the sex ratio of  $48.41 \pm 0.47$  represents the normal sex ratio for the domestic fowl, since this ratio is determined upon the total production for the first year, whereas most of the sex ratios presented in biological literature deal with ratios determined during the normal hatching season only.

## 1. THE NORMAL SEX RATIO

The term "normal sex ratio" is understood to mean the sex ratio obtained during the normal hatching season, which may be considered to include approximately the months of March and April.

Pearl (44)<sup>3</sup> has indicated the advantages, from a methodological viewpoint, of treating sex-ratio data from the standpoint of the family as the unit and, furthermore, he has shown quite clearly that the value of the male ratio, based on families of 10 or more "is much nearer the true biological norm" than values based upon the population as a whole and on families of less than 10. For instance, he obtained the following ratios: Families of all sizes,  $R\sigma = 49.45$ ; families of from one to three  $R\sigma = 55.07 \pm 2.11$ ; families of from four to nine,  $R\sigma = 49.39 \pm 0.84$ ; families of 10 or more,  $R\sigma = 48.57 \pm 0.28$ . Pearl has shown that size of family has an obvious influence on the sex ratio, and he also points out that "families under 10 can not be considered as representative of the normal fertility of the domestic fowl."

Taking Pearl's basis as the correct one on which to determine the mean sex ratio of the domestic fowl for portions of the annual production, and assuming that the value  $48.57 \pm 0.28$  was obtained for the normal hatching season, which in the State of Maine would include approximately the months of March and April, the data in this study have been arranged to show the mean sex ratio for the months of March and April and for the preceding and subsequent seasons of production, all ratios being computed on the basis of families of 10 or more.

TABLE II.—Showing the mean sex ratio for the normal hatching season and for the preceding and subsequent seasons of production. Based on families of 10 or more

Period No.	Year	Females	Males	Sex ratio	Differences
I (from the commencement of laying to the end of February)-----	1919-20	108	160	59.70 $\pm$ 1.97	I-II 7.05 $\pm$ 1.22
	1920-21	68	89	56.69 $\pm$ 1.53	
	1921-22	177	198	52.80 $\pm$ 1.18	
	Total-----	353	447	55.87 $\pm$ 0.98	
II (normal hatching season, March and April)---	1919-20	178	160	47.34 $\pm$ 0.83	II-III 12.00 $\pm$ 1.01
	1920-21	87	102	53.97 $\pm$ 2.16	
	1921-22	214	195	47.68 $\pm$ 0.93	
	Total-----	479	457	48.82 $\pm$ 0.80	
III (From the first of May to the end of October) -	1919-20	247	142	36.50 $\pm$ 0.86	I-III 19.05 $\pm$ 1.15
	1920-21	91	55	37.67 $\pm$ 1.42	
	Total-----	338	197	36.82 $\pm$ 0.61	

It should be mentioned here that the sex ratios based on the population as a whole for each of the three periods shown in Table II have been found to be as follows: Period No. I, from the commencement of laying to the end of February,  $R\sigma = 55.95 \pm 1.70$ ; Period No. II, normal hatching season, March and April,  $R\sigma = 49.01 \pm 1.03$ ; Period No. III, from the first of May to the end of October,  $R\sigma = 36.93 \pm 0.87$ . When compared with the sex ratios determined for the same periods based on families of 10 or more,  $55.87 \pm 0.98$ ,  $48.82 \pm 0.80$ , and  $36.82 \pm 0.61$ , as shown in Table II, it is apparent that the values of the ratios based on families of 10 or more show less variability.

From Table II it is evident that the sex ratio for the normal hatching season,  $R\sigma = 48.82 \pm 0.80$ , is comparable to the value,  $R\sigma = 48.57 \pm 0.28$ , as determined by Pearl. It should be remembered that Pearl was dealing with much larger numbers. The difference is not significant, however, since the difference with its probable error is  $0.25 \pm 0.85$ . At the same time, it may be pointed out that this

<sup>3</sup> Reference is made by number (italic) to "Literature cited," pp. 221-224.

is the first study to be conducted on a determination of the sex ratio in the domestic fowl based on an entire year's production, and it was practically impossible to use a large number of birds.

The most interesting fact which Table II brings out is the high sex ratio,  $R\sigma = 55.87 \pm 0.98$ , in the period of production preceding the normal hatching season and the low sex ratio,  $R\sigma = 36.82 \pm 0.61$ , in the period of production subsequent to the normal hatching season. The sex ratios for each year show a fairly regular decrease from period to period. The only apparent exception in the magnitude of decrease occurs in connection with the sex ratio in period No. II, year 1920-21, as compared with period No. I, for the same year, and this can be explained by the presence of two birds giving very high male ratios, No. 191,  $R\sigma = 91.67$  and No. 197,  $R\sigma = 66.67$ .

The difference between the sex ratios of any two periods is significant and appears to be due to specific genetic or physiological factors, the probable character of which is discussed later.

## 2. THE RELATION OF PRENATAL MORTALITY TO THE SEX RATIO

Up to the present, the problem of the sex ratio in the domestic fowl has been considered only in terms of the relation of total numbers of sexes determinable, no distinction being made between the sexes of chicks that hatched and the sexes of the dead embryos. Notwithstanding the fact that practically every egg in all the families has been accounted for, the sex ratio at hatching time does not necessarily indicate the relative proportions of the initially produced zygotes, because there may have been a differential mortality of the sexes in the embryos which died up to the twelfth day of incubation and in the embryos which died between the twelfth and twenty-first days of incubation. In other words, the prenatal mortality may have effected a deviation from the initial zygotic ratio.

It has been demonstrated that the mean sex ratio of the population as a whole, is  $48.41 \pm 0.47$  and that the mean sex ratio, based on families of 10 or more for the period from the commencement of laying to the end of February, is  $55.87 \pm 0.98$ , for the period of the normal hatching season, March and April, is  $48.82 \pm 0.80$ , and for the period from the first of May to the end of October is  $36.82 \pm 0.61$ . Two of these sex ratios show a marked deviation from equality and the problem arising out of such a situation is to determine whether a possible differential mortality has served as a cause in affecting the deviations.

The first step in an analysis of this problem is to determine, if possible, the proportions in which zygotes of the two sexes are initially produced. It is obvious that the cracked eggs and the missing eggs and chicks and probably the infertile eggs would constitute a random sample of the population as far as the sex ratio is concerned. There remain for consideration, therefore, the fertile eggs. The sex of the zygote can not be distinguished until a certain stage of embryonic development has been reached, and in this study, while the sex was determined on some younger embryos, the author felt less possibility of error when using the twelfth day as the basis of earliest determination. On the other hand, the sex could not be determined on some dead embryos in relatively advanced stages of development because of degeneration of the gonads between the time of death and the time of dissection. But the number falling under this category was very small and could not materially affect either the zygotic ratio at the time of fertilization or the sex ratio at hatching time.

Regarding the embryos which died before the twelfth day of incubation, it is impossible to determine whether or not the mortality is differential. Regarding the embryos which died after the eleventh day, accurate records have been kept, which are presented in Table III, including from the commencement of laying



to the close of the normal hatching season. The matter of bagging and recording the eggs individually on the nineteenth day of incubation in preparation for hatching requires considerably more time and trouble than simply removing the eggs from the incubators on the nineteenth day for dissecting all chicks in the shell. This is the only reason that no actual hatching was allowed to take place after the hatching season was over. It is realized now, however, that the death rate of each sex for the season subsequent to the normal hatching season might have some significance, and it is anticipated that data on this point will be made available in the near future.

Table III shows for the two periods (No. I), from the commencement of laying to the end of February, and (No. II) the normal hatching season, March and April, (1) the number of embryos dead before the twelfth day and in which sex was not determined, (2) the per cent these constitute of the total number of embryos, both dead and hatched, i. e., of fertile eggs, (3) the number of female embryos dead between the eleventh day and hatching, (4) the number of male embryos dead, (5) the number of females hatched, (6) the number of males hatched, (7) per cent males in embryos dead after eleventh day, (8) per cent males in chickens hatched, (9) per cent males of all sexes determined.

TABLE III.—*Showing the relation of prenatal mortality to the sex ratio, based on families of 10 or more*

PERIOD NO. I.—FROM THE COMMENCEMENT OF LAYING TO THE END OF FEBRUARY

Year	Embryos dying before twelfth day		Embryos dying after eleventh day		Hatched		Sex ratio of embryos dying after eleventh day	Sex ratio of chicks hatched	Sex ratio
	Number	Percent of all embryos	Females	Males	Females	Males			
1919-20.....	126	31.97	47	34	61	126	41.97±3.98	67.38±1.11	59.70±2.02
1920-21.....	75	32.33	27	27	41	62	50.00±3.74	60.19±1.24	56.69±1.53
1921-22.....	98	20.78	72	48	105	150	47.00±1.43	58.82±1.06	52.80±1.18
Total.....	299	27.20	146	109	207	338	47.74±2.02	62.02±0.74	55.87±0.98

PERIOD NO. II.—NORMAL HATCHING SEASON, MARCH AND APRIL

1919-20.....	143	29.73	65	45	113	115	45.91±2.88	50.44±1.04	47.34±0.83
1920-21.....	32	14.48	31	30	56	72	49.18±2.14	56.25±1.67	53.97±2.16
1921-22.....	87	17.74	73	45	141	150	48.13±2.53	51.55±1.51	47.68±0.93
Total.....	262	21.87	169	120	310	337	46.52±1.71	52.09±0.89	48.82±0.80

Taking Table III as a whole, four things stand out prominently: (1) The percentage of embryos dying before the twelfth day fluctuates from year to year in both periods, but for each year the percentages are lower in period No. II than in period No. I; (2) with the exception of the year 1920-21, there were slightly fewer males than females that died after the eleventh day in period No. I as compared with period No. II, the percentages for the two periods being respectively  $47.74 \pm 2.02$  and  $46.52 \pm 1.71$ ; (3) in every case there were more males than females that hatched, but for period No. I the proportion was much higher than for period No. II, i. e.,  $62.02 \pm 0.74$  as compared with  $52.09 \pm 0.89$ ; (4) in each year in period No. I the percentage of males of all sexes determined was relatively high, that for the period as a whole being  $55.87 \pm 0.98$ , while in each year except 1920-21 in period No. II the percentage of males was below fifty, that for the period as a whole being  $48.82 \pm 0.80$ .

The percentage of embryos dying before the twelfth day is of sufficient magnitude in any population so that if they were all of the same sex and if they were added to the deficient sex based on the observed ratios each observed ratio would be reversed.

In Table IV the data for periods Nos. I, II, and III are arranged to show the sex ratios required in the embryos dying before the twelfth day to produce an equality of sexes in the total population, based on families of 10 or more. On this assumption, the results in the last column of Table IV show that for the three periods there would have to be a successive increase in the percentage of males in the embryos dying before the twelfth day. Period No. III shows an extremely high assumed sex ratio of embryos dying before the twelfth day, far higher than could reasonably be expected, particularly in view of other evidence presented. Moreover, the assumed ratios of embryos dying before the twelfth day for periods Nos. I and II are each considerably different in value from the observed ratios of embryos dying after the eleventh day.

TABLE IV.—*Showing the sex ratios required in the embryos dying before the twelfth day to produce an equality of sexes in total population, based on families of 10 or more*

Period No.	Embryos dying after eleventh day		Chicks hatched		Observed sex ratios of embryos dying after eleventh day	Observed sex ratios of chicks hatched	Distribution of embryos dying before twelfth day to produce an equality of sexes in total population		Assumed sex ratio of embryos dying before twelfth day to produce an equality of sexes in total population
	Females	Males	Females	Males			Females	Males	
I.....	146	109	207	338	47.74	62.02	196.50	102.50	34.28
II.....	169	120	310	337	46.52	52.09	120.00	142.00	54.20
III.....	<sup>a</sup> 338	<sup>a</sup> 197	-----	-----	<sup>a</sup> 36.82	-----	103.00	244.00	70.31

<sup>a</sup> Includes all embryos developing up to and after twelfth day, since during this period no chicks were allowed to hatch.

It is obvious, therefore, that the number of embryos dying before the twelfth day is high enough in any population so that if a sufficient majority of the embryos were of one sex the mean sex ratio might be restored to approximate equality. The interesting feature in connection with such a supposition is that in period No. I the differential death rate would have to be considerably in favor of female survivals, since  $R\delta = 55.87 \pm 0.98$ , while in period No. II the differential death rate would have to be in favor of male survivals, since  $R\delta = 48.82 \pm 0.80$ . But the data in Table III show that the percentages of male embryos which died after the eleventh day in both periods are about the same,  $R\delta D = 47.74 \pm 2.02$  and  $R\delta D = 46.52 \pm 1.71$ . In other words, this means that from the time sex could be determined to hatching time there is a relatively higher death rate of females in both periods. Therefore, the higher death rate of females before hatching can not account for the absolute differences in the observed sex ratios between periods Nos. I and II.

In the left half of Table V the embryos dying before the twelfth day are distributed in such a way as to produce sex ratios of equality, and in the fifth column there are given the assumed ratios of these dead embryos. The assumed death rate of these embryos is in striking contrast with the observed death rate of the embryos dying after the eleventh day, as shown in the fifth and sixth columns of

Table V. Since there is no valid reason for supposing a death rate in the sexes of embryos dying before the twelfth day different from a death rate in the sexes of embryos dying after the eleventh day, it may be concluded accordingly that the death rate in the embryos dying before the twelfth day apparently does not affect the mean sex ratio differently from the death rate in embryos dying after the eleventh day. In the right half of Table V there are shown the calculated sex ratios when the death rate of the embryos dying before the twelfth day is assumed to be the same as for the embryos dying after the eleventh day. A comparison of the figures in the last two columns of Table V would indicate a relative difference in the percentage of males produced in period No. I as compared with period No. II.

TABLE V.—*Showing the results obtained when the embryos dying before twelfth day are distributed to produce an equality of sexes and on the basis of the observed sex ratios of embryos dying after the eleventh day; based on families of 10 or more*

PERIOD NO. I.—FROM THE COMMENCEMENT OF LAYING TO THE END OF FEBRUARY

Year	Em- bryos dying before twelfth day	Distribution of embryos dying before twelfth day to produce an equality of sexes		As- sumed sex ratio of em- bryos dying before twelfth day	Ob- served sex ratios of em- bryos dying after eleventh day	Distribution of embryos dying before twelfth day on basis of observed sex ratio of embryos dying after eleventh day		Calcu- lated sex ratio	Ob- served sex ratio
		Females	Males			Females	Males		
1919-20.....	126	89.00	37.00	29.36	41.97	73.42	52.58	53.95	59.70
1920-21.....	75	48.00	27.00	36.00	50.00	37.50	37.50	54.53	56.69
1921-22.....	98	59.50	38.50	39.28	47.00	51.94	46.06	52.00	52.80
Total.....	299	196.50	102.50	34.28	47.74	156.26	142.74	53.66	55.87

PERIOD NO. II.—NORMAL HATCHING SEASON, MARCH AND APRIL

1919-20.....	143	62.50	80.50	56.29	45.91	77.35	65.65	46.91	47.34
1920-21.....	32	23.50	8.50	26.56	49.18	16.26	15.74	53.28	53.97
1921-22.....	87	34.00	53.00	60.92	48.13	45.13	41.87	47.76	47.68
Total.....	262	120.00	142.00	54.20	46.52	150.12	111.88	47.48	48.82

Is there a differential death rate in the embryos dying after the eleventh day? Considering the data in Table III, it will be observed that the percentage of male embryos is lower in every case, except for the year 1920-21, in periods No. I and II, than the mean sex ratio. Pearl (44, p. 434) found the sex ratio of the dead embryos for the years 1916 and 1917 to be  $48.3 \pm 0.77$ . As between Pearl's ratio and the ratio for period No. I in Table III, the difference with its probable error is  $0.56 \pm 2.16$ , and as between Pearl's ratio and the ratio for period No. II in Table III, the difference with its probable error is  $1.78 \pm 1.87$ . Nevertheless, the data in Table III seem to show that in period No. I the death rate in embryos dying after the eleventh day does account to a slight extent for the high mean sex ratio,  $R_{\sigma} = 55.87 \pm 0.98$ . But the same interpretation can not be placed upon the data in period No. II, since the death rate is practically the same as in period No. I, but the mean sex ratio is considerably lower,  $R_{\sigma} = 48.82 \pm 0.80$ . In period No. I,  $R_{\sigma} D = 47.74 \pm 2.02$ , while in period No. II,  $R_{\sigma} D = 46.52 \pm 1.71$ . The difference with its probable error is  $1.22 \pm 2.65$  and is, therefore, of no significance. In other words, there is practically no difference in the known prenatal

mortality between period Nos. I and II. Some other explanation must be found for the differential sex ratio existing between the two periods.

Is there a differential hatching rate of the sexes in the two periods? Apparently so, as shown by a comparison of the  $R_{\sigma}H$  and the  $R_{\sigma}$  columns in Table III. The percentage of males hatching,  $R_{\sigma}H$ , is higher in every case than the percentage of males in the total population,  $R_{\sigma}$ . In period No. I,  $R_{\sigma}H = 62.02 \pm 0.74$ , while in period No. II,  $R_{\sigma}H = 52.09 \pm 0.89$ . The difference with its probable error is  $9.93 \pm 1.16$  and is equally as significant as the difference in sex ratios between the two periods,  $7.05 \pm 1.22$ , as indicated previously. It may be said, therefore, that the difference in the mean sex ratios between the two periods is affected primarily by the greater absolute number of males produced in the first period.

These observations lead to the conclusion that the differential sex ratio existing between the period from the commencement of laying to the end of February and the period of the normal hatching season can not be accounted for by a differential prenatal mortality but is accounted for by a differential hatching ratio based upon absolute numbers of the sexes produced. Such a situation rests upon a genetic or physiological interpretation.

### 3. THE RELATION OF EGG PRODUCTION TO THE SEX RATIO

The nature of the results considered so far suggest a possible correlation between egg production and the sex ratio. In order to ascertain if such is the case the records were gone over carefully and families of 10 or more were selected from each mating, based upon the antecedent egg production. The families were so selected as to eliminate the influence of prenatal mortality as much as possible, in order that each sex ratio determined might represent the highest possible proportion of its total population. The families were grouped into six representative classes, based upon the antecedent egg production. The six classes include those hens which laid from 0 to 20, from 21 to 40, from 41 to 60, from 61 to 80, from 81 to 100, and from 101 to 120 eggs, respectively, prior to selection of the families on which sex ratios were determined. (See Table VI.)

TABLE VI.—*Showing the relation between antecedent egg production and sex ratio*

Class	Antecedent production	Average antecedent production	Embryos dying before twelfth day	Females	Males	Sex ratio	Differences
1.....	0-20	4.79	25	102	173	$62.91 \pm 1.44$	$5.45 \pm 2.61$
2.....	21-40	27.86	19	94	127	$57.46 \pm 1.88$	$12.46 \pm 2.77$
3.....	41-60	47.85	18	165	135	$45.00 \pm 2.04$	$0.39 \pm 2.15$
4.....	61-80	69.45	12	108	87	$44.61 \pm 0.69$	$6.96 \pm 1.21$
5.....	81-100	88.92	16	101	61	$37.65 \pm 1.00$	$5.45 \pm 1.52$
6.....	101-120	114.50	8	80	38	$32.20 \pm 1.15$	

There are four cases in which the sex ratio deviates considerably from the normal of  $48.82 \pm 0.80$ , namely, classes 1, 2, 5, and 6. In none of these cases is the number of embryos dying before sex could be determined great enough to account for the deviation in the sex ratio. Furthermore, in the first two cases the numbers of dead embryos would have to be added to the numbers of females and in the last two cases they would have to be added to the numbers of males.

It is apparent that there is a correlation between egg production and sex ratio. Between classes 1 and 2 the difference with its probable error is  $5.45 \pm 2.61$ , between classes 2 and 3 it is  $12.46 \pm 2.77$ , between classes 3 and 4 it is  $0.39 \pm 2.15$ , between classes 4 and 5 it is  $6.96 \pm 1.21$ , and between classes 5 and 6 it is  $5.45 \pm 1.52$ . The differences are significant in every case except two between classes 1 and 2 and between classes 3 and 4. Between classes 1 and 6 the difference with its probable error is  $30.71 \pm 1.84$ , and between classes 2 and 5 the difference with its probable error is  $19.81 \pm 2.13$ . The correlation between antecedent egg production and sex ratio is high and is significant, since  $r = -0.704 \pm 0.037$ . In other words, with the birds used in this study and under the conditions of this study, the conclusion is justified that as egg production increases the sex ratio decreases.

Since it has been demonstrated that a correlation exists between antecedent egg production and sex ratio and since egg production is affected, to some extent at least, by seasonal variations, it becomes necessary to inquire concerning the possibility of a significant correlation between seasonal conditions and sex ratio. In this connection it should be pointed out that of the 45 birds, 3 commenced laying in August, 7 in September, 11 in October, 12 in November, 9 in December, and 3 in January. In view of the widely different times of the year at which the birds commenced laying, it seems logical to assume that if season affects the sex ratio birds commencing to lay early in the season as compared with birds commencing to lay late in the season should give different sex ratios for the first families of 10 or more. In order to test the validity of this assumption the sex ratios of the first families of 10 or more, obtained as nearly as possible from the time laying commenced, and according to the month laying commenced, were tabulated and averages determined. On this basis, the following average sex ratios are as follows: August, 59.17; September, 60.18; October, 61.17; November, 60.91; December, 56.67; January 58.50. It is apparent, therefore, that the season of the year has not affected the sex ratios of the first families of 10 or more.

On the other hand, if season as such affects the sex ratio, then it would be expected that in the antecedent production classes shown in Table VI, each class for which the sex ratio was determined should contain families of 10 or more produced at approximately the same season of the year. In class 1, for instance, antecedent egg production 0-20, in which  $R\sigma = 62.91 \pm 1.44$ , it should be expected that the families of 10 or more would have been produced at approximately the same season. But it is found upon examination that the following number of families occurred in the months designated: September, 5; October, 6; November, 7; December, 4; January, 3; February, 1. In class 2, antecedent egg production 21-40, in which case  $R\sigma = 57.46 \pm 1.88$ , the following number of families occurred in the respective months: December, 3; January, 6; February, 9; March, 5. These results show quite clearly that season of itself apparently does not directly affect the sex ratio, otherwise irrespective of antecedent production, families occurring at approximately the same season of the year should give sex ratios of approximately the same value.

The possible effect of season on sex ratio can be tested in still another way. On the assumption that season affects sex ratio there should be a significant correlation between the sex ratio of the families and the month in which they occur. The value of this correlation has been determined as follows:  $r = -0.165 \pm 0.076$ . This correlation is of such low value as not to be significant. Since the correlation between antecedent egg production and sex ratio is  $-0.704 \pm 0.037$ , and since the correlation between season and sex ratio is  $-0.165 \pm 0.076$ , it may be concluded that season does not affect the sex ratio unless indirectly through affecting egg production.

Crew and Huxley (6) in a study of the sex ratios in the  $F_1$  generation of a Rhode Island Red male Light Sussex female cross observes that as the season advanced the sex ratio decreased. During the spring months, February to June, the sex ratio was 50, while during August and September, the sex ratio was 42.62. The authors state as their opinion that "It is likely that this represents a significant seasonal difference, \* \* \*." In view of the results determined in the present study, however, it seems probable that the modification in sex ratio obtained by Crew and Huxley can be explained on the basis of antecedent egg production.

4. THE RELATION OF EGG WEIGHT TO SEX RATIO

It is quite conceivable that there might be a correlation between egg production and egg weight, and if such were the case then there would also be a correlation between egg weight and sex ratio. If such a correlation were manifest, however, it might or might not be significant.

The author (28), in a study of rates of growth in Barred Plymouth Rock chicks, observed a significant difference in the weights of eggs from which the females were obtained as compared with the weights of eggs from which the males were obtained. It should be explained here, however, that the only egg weights taken into consideration were those producing the chicks on which growth rates were determined, the records for analysis being selected at the conclusion of the growth period. The weights of the eggs in which embryos died and the weights of the eggs producing chicks which died prior to the termination of the period of growth studied were not considered. Moreover, it is observed that in the relatively small population of chicks used in the growth study, 38 females and 38 males, there were only 3 families of 10 or more. The difference in the weight of eggs producing the two sexes,  $2.06 \pm 0.67$ , can not be considered as evidence bearing on the problem in this study where the weights of eggs producing the two sexes throughout the first year of production have been considered, including the sexes which died between the eleventh and the twenty-first days and the sexes which hatched.

It has been noted previously that for the production years 1920-21 and 1921-22 all eggs were weighed daily as produced, and for each mating the weights have been grouped on the same basis of classification as used in Table VI. The average weights have been determined for the groups 0 to 20, 21 to 40, 41 to 60, 61 to 80, 81 to 100, and 101 to 120 eggs, as produced. Not every group for each mating consisted of 20 eggs. One hen after a long rest might lay 12 eggs in 15 days and then might not lay again for another month. In such a case the average weight of the 12 eggs was determined and allocated to the proper group, according to the antecedent production. Where hens laid more or less continuously for a long period, then the production for that particular period was divided into groups of approximately 20 eggs each. A number of hens laid more than 120 eggs, but the average weights of eggs beyond this production have not been taken into consideration because the numbers of sexes determined were so small as to prevent a true mean sex ratio being determined. The average weights classified as indicated are shown in Table VII.

TABLE VII.—*Showing the relation between egg weight and sex ratio*

Class	Antecedent production	Average egg weight	Sex ratio
		<i>Grams</i>	
1.....	0- 20	51.80±0.70	62.91±1.44
2.....	21- 40	53.91±0.69	57.46±1.88
3.....	41- 60	55.70±0.59	45.00±2.04
4.....	61- 80	55.15±0.52	44.61±0.69
5.....	81-100	55.18±0.57	37.65±1.00
6.....	101-120	53.12±0.52	32.20±1.15

It is apparent from Table VII that the average egg weight increases as egg production increases, until about 60 eggs are laid. Then the average egg weight drops slightly until about 100 eggs are laid, after which there is a more marked drop.

In a study of the nature and causes of changes in egg weight in relation to annual production in 40 Barred Plymouth Rock pullets, the author (30) has shown that the earlier in life laying commences the lower is the mean weight of the first 10 eggs laid and the lower is the mean weight of the total production. He has also shown that increase in egg weight is analogous to increase in body weight of the birds laying the eggs, and that the earlier in life laying commences the later in life both maximum body weight and maximum egg weight are attained. In another study, the author<sup>4</sup> has shown that the later in life laying commences the more nearly do the first eggs laid approach the mean egg weight of the total annual production. In other words, among the 59 Barred Plymouth Rock pullets under consideration, it has been shown that those commencing to lay as late as the month of December laid approximately as large eggs at that time as any other time during the first year of production. In view of these observations and in view of the correlation between antecedent production and sex ratio demonstrated to exist in connection with the birds used in this study, it is hardly to be expected that there should exist any significant correlation between egg weight and sex ratio based on annual production.

In Table VII it is shown that there is not more than 4 per cent difference between the average weights of any two contiguous groups, and it is hardly conceivable that such a slight difference should have any significance in this study, particularly since the percentage differences do not follow a regular order, whereas the sex ratios tend to decrease somewhat regularly. Taking into consideration the differences with their probable errors, it is evident that there is no significant relation between egg weight and antecedent egg production and certainly no correlation between egg weight and sex ratio. This point should be comparatively easy to demonstrate, however, if the weights of the eggs giving rise to the two sexes are considered.

TABLE VIII.—*Showing the relation between weights of eggs giving rise to females and males, respectively, and sex ratio*

Class	Production	Average egg weight		Difference	Sex ratio
		Females	Males		
		Grams	Grams		
1.....	0- 20	51. 37±0. 95	51. 45±0. 98	0. 08±1. 36	62. 91±1. 44
2.....	21- 40	54. 25±0. 48	54. 32±0. 52	0. 07±0. 71	57. 46±1. 88
3.....	41- 60	54. 64±0. 36	53. 93±0. 29	0. 71±0. 46	45. 00±2. 04
4.....	61- 80	55. 64±0. 51	55. 40±0. 42	0. 24±0. 66	44. 61±0. 69
5.....	81-100	54. 56±0. 99	54. 00±1. 12	0. 56±1. 49	37. 65±1. 00
6.....	101-120	52. 18±1. 21	53. 24±1. 03	1. 06±1. 59	32. 20±1. 15

The difference in weights between the eggs producing the two sexes are not regular, when the production classes are compared. For instance, in the first, second, and sixth classes the average weights of the eggs giving rise to males are slightly in excess of the average weights of eggs giving rise to females, while in the third, fourth and fifth classes the reverse is true. Furthermore, the differences in weights between the sexes are relatively slight and are not at all significant. It may be concluded, therefore, that there is no correlation between egg

<sup>4</sup> JULL, M. A. INCREASE IN EGG WEIGHT IN RELATION TO TIME LAYING COMMENCES. Amer. Nat. (In press.)

weight and sex ratio, and this is hardly to be expected, particularly in view of the fact that over 60 per cent of the absolute weight of the egg constitutes albumen and shell, both secreted around the yolk subsequent to maturation and fertilization.

5. THE RELATION OF YOLK WEIGHT TO SEX RATIO

Since the yolk is a single cell and since the earliest stages of embryonic development take place during the absorption of yolk granules in the growth of the ovum, it is well to inquire if the yolk weight bears any relation to the sex ratio. In order to study this point the weights of the eggs and their yolks laid by seven Barred Plymouth Rock hens of the poultry flock of Macdonald College were determined daily throughout one year of production. These weights are shown in Table IX, along with the sex ratios, for the production classes as shown previously, except that the sixth class is not represented owing to the limited number of eggs produced.

TABLE IX.—Showing the relation between yolk weight and sex ratio

Class	Production	Average egg weight	Average yolk weight	Sex ratio
		Grams	Grams	
1.....	0-20	46.33±0.31	15.13±0.23	62.91±1.44
2.....	21-40	50.07±0.34	15.34±0.26	57.46±1.88
3.....	41-60	52.40±0.41	15.92±0.28	45.00±2.04
4.....	61-80	51.08±0.68	15.89±0.27	44.61±0.69
5.....	81-100	49.16±0.34	15.34±0.26	37.65±1.00

It will be observed from Table IX that apparently there is a correlation between egg weight and yolk weight, the heavier the egg the heavier the yolk. This is a similar result to that observed by Atwood and Weakley, in analyzing the egg records of a number of birds at the West Virginia Experiment Station. The relationship, demonstrated in this study, existing between egg weight and yolk weight, is in close agreement with the results of Atwood and Weakley, but is in disagreement with the results observed by Riddle (46, 47) with pigeons. In a generic cross between *Turtur orientalis* and *Streptopelia alba*, the cross most largely studied, Riddle makes certain observations regarding yolk weight.

The result clearly establishes the fact that the yolks of late summer and autumn—those that produce mostly, or all, females—are larger than the yolks produced in the spring which give rise to males” (48, p. 389). “The balances alone and at once showed that the mass of the yolk of the first egg of nearly all pairs of eggs (from pure species) was less by from (usually) 9 per cent to 15 per cent than the mass of the yolk of the second egg of the pair (47, p. 350).

In regard to the first statement, the results of this study show that in the domestic fowl the yolk weight tends to increase slightly up to a production of about 60 eggs, after which it decreases. This is the same general trend followed by egg weight, and since there is no significant difference in the weights of eggs giving rise to females and males, respectively, it does not seem probable that there is any correlation between yolk weight and sex ratio. In regard to Riddle's second statement, it should be observed that the “mass” of yolk in the egg producing a male pigeon is reported to be usually from 9 to 15 per cent less than the “mass” of the yolk producing a female whereas in the domestic fowl there is no such indication of a differential yolk weight situation. Concerning yolk weights in the domestic fowl, Atwood and Weakley (1, p. 35) make the following observation: “When eggs are laid in cycles, the first egg in the cycle is usually the heaviest, the other eggs decreasing in weight according to their position in the cycle. The weight of the yolk decreases according to the position



of the egg in the cycle." The author (29) has shown that in the domestic fowl albumen weight is more highly correlated with egg weight than is yolk weight, while yolk weight is more highly correlated with egg weight than is shell weight. Also, the component parts of the egg contribute in different degrees at different times of the year toward the total egg weight. Increase in the size of yolk apparently is a direct effect of the analogous increase in the size of the body.

Finally, there is reported to be in pigeons a correlation between egg weight and yolk weight, a point which is not adequately demonstrated in the presentation of the material, and there seem to be significant correlations between both egg weight and yolk weight and sex. In the case of the domestic fowl, there is also a correlation between egg weight and yolk weight, in the material of this study found to be  $r=0.804\pm0.014$ , but there is no correlation between egg weight and sex, as shown in Table VII.

#### 6. THE RELATION OF YOLK WATER CONTENT TO SEX RATIO

In an analysis of all possible factors which might affect the sex ratio, that of the water content of the yolks of eggs might reasonably be supposed to be of minor importance. But since in an extensive series of investigations with another class of birds the factor of yolk water content is assumed to be of considerable significance, it is well to test the application of the theory in the case of the domestic fowl.

TABLE X.—*Showing the relation between yolk water content and sex ratio*

Class	Production	Average weight fresh yolk	Average weight dried yolk	Water	Sex ratio
		Grams	Grams	Per cent	
1.....	0-20	16.09	8.21	48.97	62.91 $\pm$ 1.44
2.....	21-40	16.26	8.42	48.15	57.46 $\pm$ 1.88
3.....	41-60	18.32	9.15	50.05	45.00 $\pm$ 2.04
4.....	61-80	19.81	9.88	50.13	44.61 $\pm$ 0.69
5.....	81-100	18.94	9.73	48.62	37.65 $\pm$ 1.00
6.....	101-120	18.91	9.39	50.34	32.20 $\pm$ 1.15

With two exceptions, there seems to be a slight increase in the percentage of water contained in the yolks as production increases, but the differences between any two classes are very small indeed and may not be significant. The slightly higher percentages of water content occur during the periods of production when more females than males are produced. But in the case of pigeons the reverse seems to be the case and two comments by Riddle (46, 47) deserve special notice.

The fifth correlation relates to the percentage of water in eggs of spring and autumn, and in the two eggs of a clutch. These figures for one series of analyses are given on the chart (3) last examined. They show a higher water content for the eggs of the spring (male producers) as compared with the eggs of autumn (female producers); indeed each pair of eggs from the first of the season onward has a slightly higher moisture value than the pair that follows it. The analyses further show a higher percentage of water in the first egg of the clutch than in the second in all cases. If the results of my 800 analyses all ran as smoothly as do the 8 of this series, there would be no doubt of a perfect correlation of high moisture values with small eggs; i. e., with male producing eggs—both small eggs of season and small eggs of individual clutches. But the results are not thus uniform and smooth. There are some series which seem seriously to depart from the order noted above. These can not be discussed here. We can, however, record our own belief that the situation in the chart is, in the main, indicated by the moisture determination. (48, pp. 402-403).

Chart III, referred to above, is the same as Table VII in the 1917 publication, of which Riddle says (47, p. 336):

Two additional methods of determining the amount of water in the yolks, give a satisfactory confirmation of the conclusion that the male-producing ovum contains a higher percentage of water than does the female-producing ovum.

Not only is the degree of correlation between yolk water content and sex not shown, which in 800 analyses should be readily demonstrable, but the records of percentages of water content of yolks are given for one female only. The records as given in Chart III and Table VII show 8 determinations, 2 for the month of May, 1912, 4 for the month of July, 1 for November, and 1 for December. Finally, it would appear that more data are necessary, in the case of the domestic fowl at least, before a correlation may be said to exist between yolk water content and sex ratio.

## DISCUSSION

Concerning this study, the situation seems to be as follows: (1) The sex ratio decreases from considerably above fifty in the early period of egg production to considerably below fifty in the advanced period of egg production; (2) there is no indication of reversibility of sex; (3) there is no indication of a modification of the sex ratio through external agencies, such as management and feeding of the laying stock; (4) prenatal mortality does not account for the different sex ratios observed; (5) a differential hatching rate of the sexes does account for the different ratios observed, inasmuch as it has been established that the relative proportion of male-producing to female-producing zygotes changes in relation to antecedent egg production; (6) the most reasonable explanation to account for the changes in the proportion of male-producing to female-producing zygotes seems to be differential maturation of the chromosome complexes.

### 1. THE SEX CHROMOSOMES IN THE DOMESTIC FOWL

In the case of the domestic fowl Guyer (21) described an X chromosome in the male, and in subsequent observations he (22) confirmed the original description. Guyer's observations, however, indicate that functionally the male is homozygous, which is in keeping with the apparent homozygosity of the male and the heterozygosity of the female demonstrated by breeding tests, when sex-linked characters are involved. Hance (24) has shown that the chromosome number falls within the limits of 30 to 34 and that in the male there are 2 similar chromosomes corresponding in shape and size to the largest unmated chromosome of the female chromosome complex. The results of both Guyer and Hance are in agreement in demonstrating that the male fowl is homozygous and the female heterozygous for sex, as the phenomena of sex-linked inheritance in the domestic fowl demand.

### 2. SEX RATIO DETERMINATIONS IN THE DOMESTIC FOWL

As a result of this study it has been found that the sex ratio of the domestic fowl, based on families of 10 or more, for the normal hatching season is  $48.82 \pm 0.80$ . The sex ratio based upon annual production has been found to be  $48.41 \pm 0.47$ , which is in very close agreement with the sex ratio determined for the normal hatching season. But it has also been shown that the first eggs a pullet lays tend to produce a high proportion of males and that as production increases the male ratio decreases.

There are few other statistics demonstrating the sex ratio in the domestic fowl. Darwin (7, p. 252) was probably the first to record the proportion of sexes on sufficient numbers to indicate reliability. In 1,001 Cochins reared during eight years by Stretch, as reported by Darwin, the sex ratio was 48.64, but no reference is made as to whether the proportion of sexes as given was determined at hatching time or as the fowls approached maturity. The first edition of the "Descent of Man" was published in 1871, and it is highly improb-

ble that at that time sexes would be determined at hatching time, especially if Stretch bred the buff variety of Cochins, which were so popular in England at that time. Circumstances suggest to the writer that the sex of the Cochins was determined each year as the fowls reached maturity. The next noteworthy record was observed by Field (14); in a population of 2,105 individuals the sex ratio proved to be 44.63. Thomsen (55) observed a sex ratio of 47.82 in a population of 805 Landhühner and Orpingtons. Finally, Pearl (44) in a population of 22,791 chicks, dying in embryo and hatching, of various breeds, using as a basis of determination families of 10 and over, found the sex ratio to be  $48.57 \pm 0.28$ . All of the sex ratios given in this paragraph were obtained on chicks hatched presumably during the normal hatching season.

### 3. SEX DETERMINATION DURING EMBRYONIC DEVELOPMENT

In the case of birds, where the embryo develops in the egg for some time after the sex is in all probability definitely established, it is obviously difficult to apply methods which might influence the sex after fertilization, even if it were known that such methods were really effective. With the exception of a few experiments on some of the lower animals, "there are very few cases in which there is even a suggestion that the sex of the fertilized egg can be modified by environment, and the belief that this is possible has been entirely abandoned by many of the leading investigators of the subject" (Doncaster, 12, p. 147).

Minoura (35) in an interesting study of the effects of gonad grafts in chick embryos, came to the conclusion that sexual differentiation is controllable, to some extent at least, by nongenetic factors. Using the Wyandotte breed of fowl, a small piece of testis or ovary was grafted on the chorio-allantoic membrane of developing embryos varying in age from 2 to 16 days at the time of operation. In a number of embryos between 5 and 13 days of age on which grafts grew and established vascular connections with the allantoic blood vessels, there was a modification of their reproductive systems. The modifications were of such character as to lead Minoura to conclude (35, p. 39) that "the results demonstrate that the testis and ovary produce secretions which have definite and specific physiological functions and which are capable of modifying the primary sexual characters." That it was the secretions of the gonads which produced the results indicated is established by the fact that grafts of liver, spleen, thyroid, and thymus produced no modification of the reproductive systems in the embryos on which the grafts were made. Minoura concludes, therefore, that sexual differentiation may be reversed in the chick, through the action of sex hormones secreted by the ovary and testis.

The development and differentiation of one sex is stimulated by the secretion of the gonad of the same sex and inhibited by the secretion of the gonad of the opposite sex. By means of these secretions, the differentiation of sex in the chick can be controlled to some extent. It may further be stated that the two sexes bear a quantitative and not a qualitative relation to each other (35, p. 38).

Grafts implanted on embryos which had reached a stage of about 5 days' development did not modify the embryos to any marked extent, since up to that stage the allantoic circulation has not been established. Embryos older than about 13 days are not markedly affected, since, as Minoura points out (35, p. 12), "it is probable that a resistance to foreign tissues has been developed and that such embryos are thereby enabled to destroy the grafts." This is in agreement with Murphy (41), who observed that at about the time of hatching a defensive mechanism against foreign tissues develops in the chick. Minoura secured the most marked modifications of the reproductive system in embryos which had reached the eighth and ninth days of development, when the embryo "was in a relatively early stage of sex differentiation." But Swift (53, 54) has

shown that sex is definitely established by the end of the seventh day of incubation. Furthermore, F. R. Lillie (32) has discussed quite fully the embryonic development of the ovary and testis through the indifferent period up to the period of sexual differentiation, of which he says (32, p. 395): "The sex of the embryo can first be definitely determined about the one hundred and fifty-sixth hour, by the relative sizes of the gonads, by the behavior of the germinal epithelium, and by the presence of a larger number of primordial germ cells in the germinal epithelium of the female." Minoura's statement (35, p. 34) that "this period, approximately the second week of incubation, is then the period of active sexual differentiation," does not seem entirely in keeping with Swift's statement (54, p. 407) that "at the end of the seventh day of development the sex of the individual can be easily told \* \* \*". It would seem that sexual differentiation has become definitely established by the end of the seventh day of development and that the accounts of subsequent development, as given by F. R. Lillie (31, 32), Firket (15) and Swift (53, 54), involve the further development of the sexual organs as such.

Minoura says that the result of his experiments shows "that sexual differentiation may be reversed in the chick. This result supports the explanation advanced by Lillie to account for the production of the freemartin in cattle." But in regard to the freemartin situation in cattle, F. R. Lillie and Bascom (34) say that—

In the female of cattle sex differentiation before birth is apparently due to genetic factors exclusively; in the male the genetic factors are intensified by the production of a hormone.

This situation in cattle as reported by F. R. Lillie and Bascom is similar to that in the domestic fowl as reported by Boring and Pearl (3), Morgan (39, 40), Goodale (19, 20), and Cole and Lippincott (4). The results of these investigators all go to show that the relation between the gonads and the secondary sexual characters is specific and not general. The results have demonstrated the existence of a mechanism for the control of the secondary sexual characters that is so closely associated with certain parts of the mechanism for the determination of sex that the two go together. Hormones or chalones are secreted (Boring and Pearl 2, Goodale 20, and Morgan 40) which affect the development of the secondary sexual characters, and it would have proven very interesting in this connection if Minoura had grafted pieces of testis from Brown Leghorns on the developing embryos of Barred Plymouth Rocks and reared the chicks to maturity. A reversibility of sex, as claimed by Minoura, would be exhibited if there appeared modifications of the normal sex-linked situation in the cross between Brown Leghorn males and Barred Plymouth Rock females. This is the cross that was made in the present study, and apparently there was no tendency toward reversibility of sex, in spite of the variation in sex ratio in relation to egg production.

The same objection may be offered with reference to Riddle's claims of sex reversibility in the case of pigeons. Riddle (46, p. 410) claims to have demonstrated that "germs normally female producing, have, under experiment, been made to develop males; and that germs which were prospectively male producing have been made to form female adults." But Sturtevant (52), in connection with a particular cross in which Riddle claimed to have secured an excess of female doves due to "changing over" of the males, has suggested that the probable explanation rests in the selective elimination of male-producing chromosomes during the maturation process. Furthermore, in the present study an excess of females during the periods of increased egg production is certainly not due to "changing over" of males, there being no indication whatever of a modification of the characteristic plumage color of each sex in the chicks; the plumage color

is always inherited in a definite sex-linked Mendelian manner. Also, only in very few cases did there appear to be an abnormal condition of the gonads at the time of dissection.

Two cases concerning assumed reversibility of sex have been reported recently, Crew (5) in the case of a Buff Orpington chicken and Riddle (48) in the case of a pigeon. The case reported by Crew deserves special mention, inasmuch as he placed undoubted reliance upon the statements of the original owner of the chicken concerning its apparent sex up to three and one-half years of age. The chicken is stated by Crew to have been "a purebred Buff Orpington, a good layer and a mother of chickens," although Crew makes no mention of the bird laying eggs after he secured it. It is very unfortunate, therefore, that Crew was not able to secure undisputed evidence concerning the original sex of the chicken.

Suppose, however, that the chicken was originally a female with a sex chromosome constitution of XY. Suppose also that sex reversal actually occurred, spermatozoa of XY constitution being produced, as Crew suggests. Such a bird when mated to a normal female should give a sex ratio of 33.33, since the YY chromosome complex constitution would not develop. Now, if the normal female had possessed the sex-linked barring factor, then half of the female progeny should have been black and half barred instead of all being black, as in the case of the present study. Crew was able to raise only two chickens from the mating, one male and one female, numbers too small to indicate the sex chromosome constitution of the chicken for which it is claimed the sex became reversed.

The results of the present study involving several matings and several hundred chicks from mothers possessing the sex-linked barring factor have provided no evidence of change in sex during embryonic development. Cases of reputed sex reversibility where all of the evidence may be unquestioned concerning the sex of the individual from hatching time to the time sex is supposed to have been reversed are awaited with considerable interest.

Finally, it may be said that it is possible that the sex of an embryo may be modified after fertilization if the physiological condition on which sex depends can be changed. Based upon the results of the present study the statement may be made that until more convincing evidence is available it seems necessary to conclude that sex determination is affected only at or prior to fertilization.

#### 4. SEX DETERMINATION AT FERTILIZATION

The process of fertilization is uniform in all its essentials throughout the animal kingdom. It consists in the union of two nuclei derived usually from two parents, with the mingling and division of their substance "in such a way that the chromosomes from each parent are equally represented in both the daughter nuclei produced by the first segmentation division" (Doncaster, 13, p. 117). The process of fertilization serves as about the only definite indication of the nature and function of sex. Since there are only two distinct sexes and since in any species the average ratio in which they are produced is approximately constant, it is obvious that the mechanism for producing the equal division of chromosomes is of fundamental significance. Jennings (27, p. 193) has stated the problem simply and clearly: "The really fundamental thing that mating does is to produce new combinations of hereditary characters."

The statement made above, concerning the equal representation of the chromosomes in the daughter nuclei, is of a general character, and does not apply to those cases where one sex is homozygous and the other heterozygous for the sex character. In the case of the domestic fowl, it has been shown previously from cytological observations that the female is heterozygous and the male homozygous for sex. This fact is amply supported by the breeding results secured by

a number of workers, Davenport (8, 9, 10, 11), Goodale (16, 17, 18), Hadley (23), Pearl and Surface (42, 43), Morgan and Goodale (38), Spillman (50), and Sturtevant (51). The results of these experiments involving the inheritance of sex-linked characters are of common knowledge, and their significance from the standpoint of the Mendelian inheritance of sex is so well established that nothing further regarding them will be said for the present.

It has been quite clearly established, therefore, that in the case of the domestic fowl sex is inherited, under normal conditions, as a Mendelian character. Under such conditions chance combination of the germ cells produces equal numbers of homozygotes and heterozygotes, which fact explains the approximate equality of the sexes. Sex is apparently determined at the time of fertilization and is associated with the presence or absence of a second sex chromosome.

It has been shown in this study that as egg production increases the sex ratio decreases. Pearl (45) made a similar observation as a result of an extensive study of the relation between egg production and the sex ratio during the normal hatching season. It was found that "the larger the number of eggs which a hen lays before being put into the breeding pen, the larger will be the proportion of females and the smaller the proportion of males produced by her eggs." It is regrettable that shortly after the publication of the brief note concerning the nature of his results, all of Pearl's material bearing on this problem was lost through an unfortunate accident. Apparently the decrease of the sex ratio in the present study can not be accounted for on the basis of prenatal mortality, for it has been shown that prenatal mortality affects the ratio to a slight extent only, and to the same extent throughout the year.

What factors are operative, therefore, in effecting an alteration in the sex ratio from an approximate equality of sexes which has been found to exist during the normal hatching season? There is a differential hatching rate of the sexes between the period of early egg production and the period of later production, as shown in Table III. Apparently the difference in the proportion of sexes produced from one period of egg production to another is accounted for by the difference in the absolute numbers of sexes produced from one period of egg production to another. Since in the domestic fowl the male is homozygous for sex, it does not seem probable that the spermatozoa would influence the situation, at least not from the standpoint of affecting the mechanism of the fertilization process. The fertilization process is a reaction process possessing very definite biological and biochemical characteristics, as pointed out by F. R. Lillie (33), and although the spermatozoa may provide a stimulus while the egg is still in the second maturation stage, there is no known reason why the spermatozoa might affect a change in sex determination from one period of egg production to another. Bearing on this point, an interesting case has been reported by Hays (25) where the amount of sexual service in male rabbits, which are heterozygous for the sex character, affects the sex ratio, there being a predominance of males in the first service group and then usually an increasing predominance of females as the number of services is increased. Hays has mentioned two possibilities to account for this situation:

Either female-producing spermatozoa are formed more largely than male-producing spermatozoa as the amount of service of the male increases, or the male-producing sperm are in themselves weaker than the female-producing sperm and consequently fewer of them survive to take part in fertilization.

There is no evidence on the first point, and in connection with the second point it is suggested that the female-producing sperm, because of its larger size and greater chromatin content, may be more vigorous than the male-producing sperm and thus more apt to first reach the egg for the process of fertilization. According to unpublished results of Lush, however, there is no apparent differ-

ence in size between male and female producing spermatozoa in the rabbit. The explanation offered will not hold for the domestic fowl, however, where the male is homozygous for the sex character. If there is selective fertilization, from the standpoint of male-producing or female-producing eggs being more readily fertilized under certain conditions, the only tenable explanation would seem to lie in some causes affecting the maturation of the egg.

#### 5. SEX DETERMINATION PRIOR TO FERTILIZATION

Apparently a solution of the problem concerning modifications in the normal sex ratio in the domestic fowl belongs to the domain of gamete development. It has been shown that sex is apparently not reversible during embryonic development and, furthermore, that sex is probably determined at the time of fertilization. Deviations from approximate equality of the number of males and females must be interpreted in terms of influences affecting the development of the gametes which subsequently take part in the process of fertilization.

Along this line must first be mentioned the possibility of differential maturation, since it has already been observed that during the early period of egg production the actual number of male-producing zygotes exceeds the actual number of female-producing zygotes, and that as egg production increases the situation tends to be reversed. The possibility of the existence of differential maturation has been shown to exist in some forms of insects. In the case of the currant moth, *Abraxas grossulariata*, Doncaster (12) bred seven generations of a strain of *Abraxas* in which some broods in each generation were all females. Doncaster (12, p. 132) suggests the possibility that "in these families all of the eggs have a tendency to extrude the male-determining sex chromosome, just as in aphids the male-producing eggs all extrude the female-producing X chromosome." In *Talaeoporia tubulosa*, Seiler (49) has shown that in the anaphase of the first polar division the unpaired X chromosome seems to go into the polar body rather more frequently than into the oocyte nucleus. In this species females are more numerous than males, so that genetic and cytological facts are in agreement.

Might a similar condition exist in the case of the domestic fowl? The process of maturation begins in the fully ripe follicle and is completed after ovulation. At the time of ovulation the first maturation spindle is still in the equatorial plate stage, the outer end of the spindle being in almost immediate contact with the surface of the ovum. The first polar body is thrown off and almost immediately the second maturation spindle is formed. The position of the second maturation spindle is similar to that of the first, and a second polar body is thrown off. Whichever maturation division serves as the reduction division must also serve in the elimination of a greater number of female-producing than male-producing complexes during the early period of egg production and the elimination of a greater number of male-producing than female-producing complexes during the later periods of egg production, if this is the explanation of the results obtained. During the normal hatching season there would seem to be no influence at work affecting the apparent chance elimination of either kind of complex. Is there any evidence concerning the selective elimination of complexes of a particular kind? Heape (26) has thus interpreted his results with canaries bred under different conditions. He found that certain conditions affected the sex ratio, and he assumed that under conditions of early breeding and light feeding male-producing gametes are produced in excess, while under conditions of late breeding and heavy feeding female-producing gametes are produced in excess. Heape's results are similar in nature to those secured by

Pearl (45), Riddle (46, 47), Crew and Huxley (6), and the results secured in this study, except that in the last four cases the method of feeding has apparently had no influence, inasmuch as the birds were all fed similarly throughout the duration of the tests. In the case of the present study, then, if differential maturation occurred it must be accounted for as a result of conditions of egg production. That is, differential maturation must be looked upon as an effect, produced by certain conditions, the nature of which will be discussed presently.

A very interesting case, bearing on the discussion as it has been developed up to the present, is reported in one of the forms closely related to the plant lice *Phylloxera fallax*. Morgan (36, 37) has shown that the sex of the individual developing from the parthenogenetic egg is determined before the distribution of sex chromosomes at polar body formation. This is explained by the fact that the male-producing and female-producing eggs are already differentiated from one another before the time of polar body formation. The eggs which will develop into males through the elimination of the double sex chromosome are smaller than the female-producing eggs, where there is no elimination. In this case, therefore, the elimination of the sex chromosome is not a matter of chance but is determined by conditions antecedent to the time of polar-body formation. Such conditions, therefore, constitute a sex-determining factor in the chain of causation of sex.

So it would seem to be in the case of the domestic fowl. As to the nature of these conditions, hardly more than speculative suggestions can be offered, but perhaps they will not be less speculative than other theories advanced to account for the manner in which sex is determined.

It would seem that many of the more general statements implying that sex determination is readily modified by external conditions and that sex may be controlled are not in harmony with all the facts. The approximate equality of the sexes is one of the outstanding phenomena among the higher forms which reproduce bisexually. But if environmental factors play such a highly significant part in the modification of sex determination as is so frequently claimed, is it not almost inconceivable that with so many organisms under such varying environmental conditions throughout all parts of the world the sexes should usually approximate equality? Furthermore, it may be legitimate to inquire as to the nature of sex potencies which are assumed to be operative in producing sex and what causes contribute to the development of different degrees of potency. It is now apparent that in some of the experiments designed to test the stability of the sex-determining mechanism many of the interpretations based upon the results secured require examination.

A number of factors have been found to be associated with one sex or the other, and the results secured in several lines of work would seem to be materially weakened by the apparent desire to disregard, in a large measure, the significance of the chromosomal mechanism, which is continually receiving stronger and stronger support from cytological investigations. It should not be overlooked that notwithstanding the laborious procedure and technical difficulties involved in conducting cytological investigations remarkable achievements have been accomplished in definitely correlating cytological facts with genetic results for an ever increasing number of forms. If the different phenomena which have been found to be associated with one sex or the other can also be shown to be associated with the constitution of the chromosome complex then it would appear that the metabolic and chromosome theories can be harmonized and real progress made toward a solution of the problem of the causation of sex.

As far as the chromosome theory of sex is concerned, in view of the variety of circumstances under which sex ratios have been modified and the mode of sex



determination apparently affected, and also in view of the most recent interpretations concerning the physiological constitution of the chromosomes, with their associated interactions, it seems certain that the simple hypothesis of an unchangeable hereditary entity must be modified. Two views may be expressed as follows: The sex chromosome may cause sex, or the presence or absence of the sex chromosome may be the effect of the two sexes being produced by differing levels of metabolism. At the same time, in the case of the domestic fowl, as in many of the other higher forms, the presence or absence of a second sex chromosome seems to be definitely associated with sex. But it is evident that sex can not depend on a chromosome alone, for the chromosome obviously acts by its relation with the cell protoplasm. Seemingly the presence of a particular chromosome provides only one side of a reciprocal reaction, while the determination of sex must depend upon the complete result of all the reciprocal reactions.

There may not be any heredity other than Mendelian, and although it is evident that the chromosomes are responsible for the segregation of Mendelian characters, it has become apparent that the cytoplasm can not be relegated to a position of serving as a medium only in which the chromosomes carry on their reactions. The chromosomes can not be regarded as acting independently of the cytoplasm and in fact there is much evidence of interaction between chromosomes and cytoplasm. The coexistence in the primary oocyte of the germinal vesicle and the enormous growth of the cell with its formation of reserve food material suggests a causal connection between them. The diffusion of chromatin in the ordinary resting nucleus has the result of increasing its area in proportion to its mass and thus favors active metabolism. Similarly, it has been held that the excessive diffusion of chromosomes in the germinal vesicles of many animals gives rise to an intense activity of the chromosomes in the elaboration of yolk. Finally, it may be said that the interaction which takes place between nucleus and cytoplasm is certain to effect a modification in both nucleus and cytoplasm.

Applying these ideas to the case in hand, it does not seem unreasonable to suppose that the nature of the reactions within the nucleus would vary from time to time throughout the maturation process. Variations in the chemical composition of the eggs in relation to increased egg production in the domestic fowl which have been shown to exist, might also exert an influence on the nature of interactions between the chromosomes and the cytoplasm. It is quite conceivable, therefore, that during the early period of egg production a given condition of reactions might prevail, and this particular condition might be modified as egg production increased. This being possible, it is also possible that under a given set of conditions certain factors may operate in causing the female-producing complex to be extruded more frequently in polar-body formation than the male-producing complex, and conversely when the conditions become altered. If this be possible, then it is reasonable to suppose that during the early period of egg production in the domestic fowl female-producing complexes are extruded more frequently than male-producing complexes and that as production increases the situation becomes reversed. Finally, since it seems obvious that there are more male-producing than female-producing zygotes produced during the early period of egg production and that this situation becomes reversed as egg production increases, and since it therefore follows that there must, of necessity, be differential maturation, this hypothesis has been suggested to account for more males than females being produced during the early period of egg production and more females than males during the later period of egg production.

# SUMMARY

1. In the case of the domestic fowl, there is found to be a significant negative correlation between antecedent egg production and the sex ratio.
2. The sex ratio of the three yearly populations is  $48.41 \pm 0.47$ .
3. The sex ratio, based on families of 10 or more, for the normal hatching season, is  $48.82 \pm 0.80$ .
4. With the annual egg production divided into classes of 20 eggs each, the sex ratio, based upon antecedent production, is as follows: 0-20,  $62.91 \pm 1.44$ ; 21-40,  $57.46 \pm 1.88$ ; 41-60,  $45.00 \pm 2.04$ ; 61-80,  $44.61 \pm 0.69$ ; 81-100,  $37.65 \pm 1.00$ ; 101-120,  $32.20 \pm 1.15$ .
5. The correlation between antecedent egg production and sex ratio is  $-0.704 \pm 0.037$ .
6. The correlation between season and sex ratio is  $-0.165 \pm 0.076$ , and is probably incidental.
7. There is no apparent correlation between egg weight and sex ratio.
8. There is no apparent correlation between yolk weight and sex ratio.
9. There is no apparent correlation between yolk water content and sex ratio.
10. The feeding and general management of the birds having been practically identical for the three years, it is obvious that general environmental conditions have not affected the sex ratio.
11. The variation in the sex ratio in relation to egg production can not be accounted for on the basis of prenatal mortality.
12. There is no indication of reversibility of sex, this possibility having been tested by using the dominant sex-linked barring character of Barred Plymouth Rocks with the recessive plumage color character of Brown Leghorns.
13. It is apparent that male-producing zygotes are produced in excess of female-producing zygotes during the early period of egg production and that as egg production increases female-producing zygotes tend to be produced in excess.
14. An hypothesis is developed to account for differential maturation, which in turn would account for the modified sex ratios.

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# A GRADIENT OF PERMEABILITY TO IODIN IN WHEAT SEED COATS<sup>1</sup>

(A PRELIMINARY NOTE)

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Statements in literature on the relation of semipermeable seed coats to the entrance of toxic solutes, expressed as recently as 1923 (Harrington and Crocker),<sup>3</sup> are to the effect that a solute like iodine enters only to a small degree through the general surface of the grain, but largely through the hilum or micropyle whence it spreads laterally and in a distal direction under the seed coats. Experiments by the writer point to contrary conclusions as follows: (1) there is very little spread of iodine under the seed coats from the embryo end either laterally or in a distal direction; (2) this is equally true for all other parts of the seed; (3) entrance of iodine takes place over the entire surface of the grain radially but not uniformly; (4) the *apparent* lateral and distal spread from the embryo end is due to the existence of a gradient of permeability to iodine in the seed coats, which permeability is greatest near the embryo end, diminishes to a minimum near the distal end and again increases slightly. These conclusions are supported by experiments with wheat seeds coated in various ways with waterproof substances like paraffin. The experiments have shown that (1) when the embryo end only is exposed, iodine penetrates only as far as the limits of the exposed area and fails to spread much further laterally or distally under the seed coats protected by paraffin; (2) the same is true for any exposed region of the kernel partially paraffin-coated, the stain only goes as far as the limits of the exposed part; (3) when the embryo end alone is coated, the entrance of iodine manifests itself progressively, beginning always on the exposed part nearest the embryo end 180 degrees from the grooved surface and continuing in a lateral and distal direction but not toward the coated embryo end; and the blackening due to iodine at any point on such a grain corresponds in degree with that shown after a similar period of time on the same point of a wholly unprotected grain.

Ultimately, the entire grain is softened by entrance and spread of water from the iodine solution but is not discolored except near the exposed surface. This indicates that the failure of iodine to spread rapidly laterally and inwardly is due to its removal either (1) by chemical combination with the starch nearest the point of entry, or (2) by adsorption (Biltz)<sup>4</sup> on the starch immediately underneath the exposed area, while water goes through.

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<sup>2</sup> The writer is deeply indebted to Prof. R. A. Harper, of Columbia University, in whose laboratory the greater part of the experiments were made.

<sup>3</sup> HARRINGTON, G. T., and CROCKER, W.—STRUCTURE, PHYSICAL CHARACTERISTICS, AND COMPOSITION OF THE PERICARP AND INTEGUMENT OF JOHNSON GRASS SEED IN RELATION TO ITS PHYSIOLOGY. *Jour. Agr. Research* 23: 193-222, illus. 1923.

<sup>4</sup> BILTZ, W.—UEBER DIE BLAUE ADSORPTIONSVERBINDUNG VON BASISCHEM LANTHANACETAT UND IOD. *Ber. Deut. Chem. Gesell.* 37: 719-724. 1904.

The removal of iodine by the nearest starch layer was corroborated by experiments with three-eighths inch cubes of dry starch gels, placed in iodine solution. These became completely soft through entrance of water but remained colorless except for a blackened surface layer which was narrow and fairly well delimited in cross-section.

Microtome sections of immature wheat seeds fixed in Flemming's strong solution showed near the distal end a deep blackening of the cutin layer (more strongly cutinized in the mature seed) with scarcely any discoloration at the embryo end, and a gradation of color between. The greater degree of cutinization first demonstrated by osmic acid in a direction opposite to the permeability to iodine was subsequently found in the mature seed by the use of Flemming's triple stain and this makes it certain that the cutinization gradient remains nearly the same in the mature seed, although this may be obscured owing to the higher degree of cutinization attained in the entire surface layer. This indicates a cytological basis for the gradient of permeability experimentally observed.

# FURTHER STUDIES ON THE RELATIVE SUSCEPTIBILITY TO CITRUS CANKER OF DIFFERENT SPECIES AND HYBRIDS OF THE GENUS CITRUS, INCLUDING THE WILD RELATIVES<sup>1</sup>

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## INTRODUCTION

The object of this paper is to present in a summarized form the results of the writer's investigations, extending from 1917 to 1921, on the relative susceptibility of citrus canker of a large number of different species, hybrids, and relatives of the genus *Citrus*. During the progress of this investigation two detailed reports<sup>2,3</sup> have appeared. Since the last report was issued, new plants have been added to the experiments, further numbers successfully infected and in some cases continued observations have modified previous conclusions.

The reader is referred to the reports already published for the description of the plants used, the location of the experiments, experimental methods employed and the detailed observations made. The complete data on each plant included in the experiments during the course of the investigation are on file in the Office of Crop Physiology and Breeding Investigations, Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.

## SUSCEPTIBILITY OF THE WILD RELATIVES OF THE GENUS CITRUS

Repeated attempts to infect *Melia azedarach* L., in the field were unsuccessful. Likewise, under greenhouse conditions, negative results were obtained with *Xanthoxylum bungei* Planch., *Xanthoxylum* sp., *Toddalia asiatica* (L.) Kurz and *Glycosmis pentaphylla* Correa. A few small infections on leaves were obtained under greenhouse conditions with *Claucena lansium* (Lour.) Skeels, and *Chalcas exotica* (L.) Millsp. However, these results could not be duplicated in the field. *Casimiroa edulis* Lav. & Lex., was the only plant of this group that was successfully infected at wounds on the leaves, in the field and greenhouse.

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<sup>2</sup> PELTIER, G. L. SUSCEPTIBILITY AND RESISTANCE TO CITRUS-CANKER OF THE WILD RELATIVES, CITRUS FRUITS, AND HYBRIDS OF THE GENUS CITRUS. [Preliminary paper.] Jour. Agr. Research 14: 337-358, illus. 1918.

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*Chaetospermum glutinosum* (Blanco) Swingle was quite easily infected both in the greenhouse and field in the absence of wounds. In fact, natural infection from adjoining plants in the field frequently occurred. Judging from its susceptibility under southern Alabama conditions, it is as susceptible as some of the Citrus fruits. However, these plants can not grow in southern Alabama as they are very easily killed by the winter temperatures generally prevailing in this region. Of the remaining plants belonging to the subtribe Aeglinae, *Balsamocitrus dawei* Stapf and *Aeglopsis chevalieri* Swingle remained free from infection after repeated attempts to infect, while *Aegle marmelos* Correa, although infected at wounds on the leaves in the greenhouse, remained nonsusceptible in the field.

*Feronia limonia* (Correa) Swingle was infected to some extent at wounds in the greenhouse. No results were obtained in the field. In the case of *Feroniella lucida* Swingle, however, positive results were obtained both in the greenhouse and field.

On the plants in subtribe Lavanginae, two species, *Triphasia trifolia* (Burm.) P. Wilson and *Severinia buxifolia* Ten. proved to be strictly nonsusceptible in the field and greenhouse, while *Paramignya monophylla* Wight was easily infected in the greenhouse. Likewise, plants of *Hesperethusa crenulata* (Roxb.) Roem. were easily infected in the greenhouse and field through natural infection from adjoining plants.

In the case of *Severinia buxifolia* it was repeatedly observed that the leaves, petioles, and stems when punctured or wounded had the ability to form cork tissues very rapidly. Owing to the nonsusceptibility of *Severinia buxifolia* to citrus canker and the fact that it thrives under southern Alabama conditions, its propagation as a hedge plant to replace *Poncirus trifoliata* (L.) Raf. should be encouraged. Incidentally, it may prove suitable as a stock for some types of Citrus fruits.

All plants of the subtribe Citrinae were successfully infected, but in varying degrees. Attempts to infect *Citropsis schweinfurthii* Swingle and M. Kellerman in the field were unsuccessful, although small unruptured spots were produced at wounds in the greenhouse. The two species of *Atalantia* have been rather easily infected. Likewise, plants of *Eremocitrus glauca* (Lindl.) Swingle were quite easily infected both in the greenhouse and field.

The three species and one variety of *Microcitrus* and *Eremocitrus glauca* have proved susceptible enough under controlled conditions to be naturally infected in their native habitat, if a source for infection was present. Both of these genera are native of the east coast of Australia, and while citrus canker has only been reported from the northern territory of Australia, should canker be introduced at any time into the Citrus districts of Queensland, there is a bare possibility that citrus canker might be disseminated to the native growths of *Microcitrus* and *Eremocitrus*.

*Fortunella hindsii* (Oliver) Swingle is rather susceptible; much more so than any of the other three species of kumquats. While these were all successfully infected in the greenhouse, only two, *F. japonica* (Thunb.) Swingle and *F. Margarita* (Thunb.) Swingle gave positive results in the field.

In the spring of 1918, thirty 3-year-old trees of *Fortunella margarita* budded on *Poncirus trifoliata* were planted in the isolation field. From the summer of 1918 through the season of 1921, not a single canker spot was found on these trees, notwithstanding the fact that various methods of inoculations were tried and that during the active growing season they were at all times exposed to natural infection. The kumquats, with the exception of *F. hindsii* are highly resistant to Citrus canker and it is only under the most favorable field conditions and through wounds on the leaves that they can be infected.

In conclusion, it might be stated that so far as the menace of citrus canker to the Citrus industry in the United States is concerned, with the exception of *Poncirus trifoliata*, none of the relatives of Citrus, native or introduced, discussed

above are sufficiently susceptible to warrant further attention. However, it is of scientific interest to know that *Pseudomonas citri* is not limited in its attack to the genus Citrus but can produce, under certain conditions, infections on a wide range of plants in the family Rutaceae to which Citrus belongs. In this connection, the plant breeder is furnished facts which may assist him in developing hybrids of a commercial nature which are resistant to citrus canker.

### SUSCEPTIBILITY OF CITRUS FRUITS

The difference in the susceptibility of the two forms of *Citrus hystrix* DC has been consistent throughout the investigation. The round leaf form equals grapefruit in its susceptibility to canker, while the pointed leaf form shows some resistance to canker. Various forms of this obscure but large group should be tested further in the Philippine Islands where it is native, and undoubtedly, forms resistant to citrus canker will be found.

No changes were noted in the reaction of the citrons, lemons, and limes tested in the greenhouse. None of these plants were used in the field, so that their susceptibility to canker must be based on greenhouse results. While plants of the Ichang lemon were tested both in the greenhouse and field, no changes have occurred to modify previous reports.

All grapefruit plants tested are susceptible to citrus canker. Of the pummelos, the Hirado Buntan and Siamese have shown some resistance in that infection was almost wholly confined to the leaves with only an occasional spot on a young, growing twig.

Of the sweet orange group, no variety or species stands out as resistant, although they are not as susceptible as some of the other citrus fruits.

All of the plants belonging to the *Citrus nobilis* group show resistance to canker. In the majority of instances, canker was limited to scattering spots on the young leaves and on an occasional twig. The Cleopatra tangerine, which appears to be an excellent stock for some types of Citrus might after further trials be found suitable to replace the trifoliolate orange as a stock.

As has been pointed out in a previous report, Tanaka<sup>4</sup> and Scott<sup>5</sup> have shown that there are several distinct strains of Satsuma grown in southern Alabama. The two most commonly found are the Owari and Ikeda. Scott describes them as follows:

The fruits of the Owari are flat, thin skinned, depressed at both the blossom and stem ends, and practically seedless, maturing in Alabama the latter part of October or first of November. The leaves are very broad, particularly at the base, and the tree has an upright habit of growth.

The fruits of the Ikeda variety are not as flat as those of the Owari, are not depressed at either the stem or blossom end, have a very coarse texture and a thick skin, and generally contain a few seeds. The season of the Ikeda is three or four weeks later than that of the Owari variety. The leaves are narrow, and the tree has a very spreading habit of growth.

From various sources reports of a difference in susceptibility between these strains were received, and in order to determine if they varied in susceptibility the following experiment was conducted.

During the early spring of 1918, thirty 3-year-old trees budded on *Poncirus trifoliata* of the Owari strain and a like number of the Ikeda strain were planted in the isolation field. As a control, thirty 3-year-old Duncan grapefruits budded on *Poncirus trifoliata* were planted between the two rows of Satsumas. Observations were made as to the number of trees infected and the parts of the plants attacked at intervals during the growing season over a period of three years. During this time the number of trees did not remain the same in that a number died or were cut out to make room for the remainder to grow. At the conclusion of the test, only 13 of each kind of tree remained. Since these trees were always surrounded by susceptible trees, natural infection was depended on entirely.

<sup>4</sup> TANAKA, T. VARIETIES OF THE SATSUMA ORANGE GROUP IN JAPAN. U. S. Dept. Agr. Bur. Plant Indus. Crop Physiol. and Breeding Inves. C. P. & B. I. Circ. 5, 10 p., illus. 1918.

<sup>5</sup> SCOTT, L. B. VARIETIES OF THE SATSUMA ORANGE GROUP IN THE UNITED STATES. U. S. Dept. Agr. Bur. Plant Indus. Hort. and Pomol. Inves. H. & P. Circ. 1, 7 p., 1918

At the conclusion of the investigation, no distinct difference in the susceptibility of the two strains of Satsumas was apparent. While the number of Ikeda trees infected earlier in the season was greater than the Owari, the reverse occurred later in the season, so that more of the Owari trees were infected than Ikeda. Further, infection was somewhat lighter on Owari than Ikeda although the percentage of Owari trees infected by canker was larger than that of Ikeda. Thus, from the results of this experiment conducted with a fair number of plants over a period of three years, absolutely no differences in the susceptibility of these two strains was apparent. With only a few exceptions, canker was limited to the young growing leaves and an occasional twig. On the other hand, the grapefruit plants were very badly infected at all times during the season, canker being found on the leaves, thorns, and round wood.

*Citrus mitis* Blanco proved to be more resistant than the Satsuma under field conditions. Canker at all times was limited to a few young leaves and an occasional twig. In the greenhouse experiments, the plants were more susceptible as twig infection was common and occasionally the round wood was attacked.

Likewise, the Kansu orange proved to be decidedly resistant. Very few canker spots on the leaves and no twig infection were noted. Of the other Citrus fruits tested, all proved to be susceptible.

In the continued search among the Citrus fruits for promising canker resistant types, the search will have to be confined to a few groups which show some promise of being resistant to canker.

#### RELATIVE SUSCEPTIBILITY OF THE CITRUS HYBRIDS

Both faustrime and faustrimon are more susceptible than *Microcitrus australasica* (F. Muell.) Swingle. Faustrimedin is not as susceptible in that infection is limited to a few spots on the leaves and an occasional twig.

All of the *Poncirus trifoliata* crosses, including citranges, citrumelo, citradia, citrandarin, citrunshu, cicitrange, and citraldin, are susceptible, and in no instance can any one number be selected as showing enough resistance to citrus canker to warrant its use as a canker resistant stock or orchard tree. While none of these hybrids are resistant to canker, several of them stand out above the common level and may be of some use for further breeding and crossing purposes. Throughout this investigation the Willets citrange has consistently shown less infection than any of the other citranges. The fact that it is one of the parents of the citrangequat, the most resistant hybrid so far found, substantiates to some extent these results.

On the whole, it can be stated that when *Poncirus trifoliata* is crossed with any of the more resistant Citrus species the resistance of the progeny is lessened.

Of the four citrange crosses, citrangequat, citranguma, citrangarin, and citrangedin, it happens that the second parent in each case is resistant to canker. Unfortunately, no hybrids were tested with a susceptible parent, so that we do not know what is the direct influence of the citrange parent on canker susceptibility. The citrangequat has consistently proved very resistant, in fact, as much so as its second parent. It appears to be the most promising canker resistant hybrid so far found. One disadvantage in the use of citrangequat as a stock has been the small number of seeds produced. However, Swingle and Robinson<sup>6</sup> have recently developed a method of propagation by the use of root cuttings, so that stock of this hybrid can now be rapidly developed. If it is found that it will make as desirable a stock as *Poncirus trifoliata*, it can replace the trifoliolate orange, which at the present time is a menace to the final eradication of citrus canker in the Gulf Coast States. The other three citrange crosses, while they do not show as much

<sup>6</sup> SWINGLE, W. T., and ROBINSON, T. R. TWO IMPORTANT NEW TYPES OF CITROUS HYBRIDS FOR THE HOME GARDEN—CITRANGEQUATS AND LIMEQUATS. Jour. Agr. Research, 23: 229-238, illus. 1923.

resistance as the citrangequat, are sufficiently resistant to warrant further trials both as a stock and as orchard trees.

Limelo, orangelo, satsumelo, clemelo, siamelo, and tangelo, hybrids with grapefruit as one parent, vary in their susceptibility to canker. In no instance are any of these hybrids as resistant as Satsuma to citrus canker, although a few numbers are only slightly more susceptible. Because of the fact that one of these hybrids, the tangelo, is being grown commercially and has merits which are demanding attention from the orchardists and the public, the reaction of these hybrids to canker is of importance. Until further information is at hand it is safe to state that the tangelo and satsumelo, with some numbers of siamelo, show enough canker resistance to place them beside the mandarin oranges in their resistance to canker.

Likewise, the limequat and orangequat can be regarded as canker resistant as Satsuma. The orangequat has been observed very closely in the field because of its apparent similarity to the Satsuma, both in the type of growth and in its reaction to citrus canker and scab. Its relation to the Satsuma, if there is any, will not be known until fruit of the orangequat is produced and compared with Satsuma. Oranguma and siamar are slightly less resistant than the above hybrids.

Judging from the results obtained with the various hybrids of *Citrus mitis*, it appears while somewhat resistant itself, it does not carry this resistance in the hybrid. Thus, in the order of their susceptibility, we have sopomaldin, citraldin, calarin, and calashu, which are equally as susceptible as the other parent, grapefruit, trifoliate orange, tangerine, and Satsuma, respectively.

In the search for promising canker-resistant plants, the results of over four years' investigations seems to point to the fact that our best plants will come from the hybrids. Already one or two of the hybrids showing almost as much resistance as Satsuma have been set out in a small way. No doubt, as the result of further tests by the Office of Crop Physiology and Breeding Investigations, Bureau of Plant Industry, United States Department of Agriculture, other canker-resistant hybrids will be found.

## DISCUSSION

In Tables I, II, and III we have listed each number tested, giving the type and number of plants used, its index of susceptibility, and the part or parts of the plant attacked. The index of susceptibility is the maximum that the plant has shown while it was under investigation. The same is true of the parts of plants attacked. Thus, the list as it stands records the maximum susceptibility shown by the plants of each individual number.

It must also be remembered that for the most part these plants consisted of small seedlings of budded plants. In only a few cases were the plants large enough to bloom and set fruit. Further, they were fertilized to stimulate new growth, for it was only on the new growth that canker developed in the majority of cases. Also during the greater part of the work many of the more susceptible plants were badly infected with canker, so that an epidemic condition existed both in the greenhouse and field, and consequently natural infections could be counted on at all times. Overwintering of canker in the field occurred each season, so that during the last three seasons very few of the plants were inoculated.

In all of our publications on citrus canker one point has been continually stressed, namely, that the plants must be in good growing condition for canker to infect. In other words, the plant must be making a good, vigorous growth to produce maximum infection. Therefore every means was used to keep the plants in a good, vigorous growing condition during the investigation.

Under grove conditions with older trees no doubt many of the numbers tested would show greater resistance to canker. However, the fact that under the conditions existing maximum susceptibility was obtained renders the results more valuable in that it gives us a truer index of susceptibility.

TABLE I.—Relative susceptibility of the wild relatives of *Citrus* to canker

Genus and species	C. P. B. No. <sup>a</sup>	Type of plant	Location	Number of plants	Results
<i>Rutaceous plants not closely related to the genus Citrus</i>					
<i>Melia azadarach</i> .....	Alabama	Seedling	Field	6	Negative.
<i>Xanthoxylum bungei</i> .....	11180	do	Greenhouse	1	Do.
<i>Xanthoxylum sp</i> .....	11269	do	do	1	Do.
<i>Casimiroa edulis</i> .....	7923	do	do	3	Leaves only at wounds.
		do	Field	1	Do.
<i>Toddalia asiatica</i> .....	11145	do	Greenhouse	1	Negative.
<i>Glycosmis pentaphylla</i> .....	2905	do	do	2	Do.
		do	Field	2	Do.
<i>Clauцена lانسium</i> .....	7936	do	Greenhouse	2	Leaves only at wounds.
		do	Field	4	Negative.
<i>Chalcas exotica</i> .....	7975A	do	Greenhouse	2	Leaves only at wounds.
	7980	do	Field	7	Negative.
<i>Tribe Citreae, subtribe Aeglinae</i>					
<i>Aegle marmelos</i> .....	7759	do	do	2	Do.
	7983	do	Greenhouse	2	Leaves only at wounds.
<i>Aeglopsis chevalieri</i> .....	7633	do	do	1	Negative.
		do	Field	1	Do.
	7772	Cutting	Greenhouse	2	Do.
<i>Chaetospermum glutinosum</i> .....	7138	Seedling	Field	2	Susceptible minus.
	7799	do	Greenhouse	-----	Do.
		do	Field	-----	Negative.
	7814	do	Greenhouse	-----	Susceptible minus.
		do	Field	-----	Negative.
<i>Balsamocitrus dawei</i> .....	2920	Budded <sup>b</sup>	Greenhouse	1	Do.
		do	Field	1	Do.
	7725	Budded <sup>c</sup>	do	1	Do.
<i>Subtribe Feroninae</i>					
<i>Feronia limonia</i> .....	2763	Seedling	Greenhouse	3	Leaves only, mostly at wounds.
		do	Field	5	Negative.
	11287	do	do	2	Do.
<i>Feroniella lucida</i> .....	7882	do	Greenhouse	2	Leaves only, mostly at wounds.
		do	Field	7	Do.
<i>Subtribe Lavanginae</i>					
<i>Hesperethusa crenulata</i> .....	2758	do	do	4	Susceptible minus.
	2759	do	Greenhouse	2	Do.
		do	Field	4	Do.
<i>Triphasia trifolia</i> .....	2689A	do	Greenhouse	2	Negative.
		do	Field	2	Do.
	7780	do	Greenhouse	1	Do.
<i>Paramignya monophylla</i> .....	7874	do	do	1	Leaves easily infected.
<i>Severinia buxifolia</i> .....	2760	Cutting	do	2	Negative.
		do	Field	17	Do.
	11322	Seedling	do	12	Do.
<i>Subtribe Citrinae</i>					
<i>Citropsis schweinfurthii</i> .....	7999	do	do	2	Negative.
	11260	do	Greenhouse	2	Leaves only at wounds.
		do	Field	5	Negative.
<i>Atalantia citrioides</i> .....	7534	Cutting	Greenhouse	4	Leaves easily infected.
		do	Field	4	Do.
<i>Atalantia ceylonica</i> .....	11225	Seedling	Greenhouse	1	Leaves and stems easily infected.
<i>Poncirus trifoliata</i> .....	Alabama	do	do	4	Extremely susceptible.
		do	Field	24	Do.
<i>Eremocitrus glauca</i> .....	7239	do	Greenhouse	4	Susceptible minus.
		do	Field	9	Do.
<i>Fortunella margarita</i> .....	7597	do	Greenhouse	3	Leaves only, resistant.
	11302	do	Field	3	Do.
	11327	do	do	2	Negative.
<i>Fortunella japonica</i> .....	11301	do	Greenhouse	3	Leaves only, resistant.
		do	Field	2	Do.
<i>Fortunella crassifolia</i> .....	7946D	do	do	3	Negative.
	11047	do	Greenhouse	6	Leaves only, resistant.
	11047A	do	Field	3	Negative.
<i>Fortunella hindsii</i> .....	11046A	do	Greenhouse	4	Susceptible minus.
		do	Field	8	Do.
	11319	do	do	6	Do.
<i>Microcitrus australasica</i> .....	7600	Cutting	Greenhouse	3	Do.
		Seedling	Field	5	Do.
	7600B	do	Greenhouse	2	Do.
		do	Field	5	Do.
<i>Microcitrus australasica sanguinea</i> .....	7775B	do	Greenhouse	1	Do.
<i>Microcitrus garrowayi</i> .....	11008	Cutting	do	3	Do.
		do	Field	2	Do.
<i>Microcitrus australis</i> .....	7307	do	Greenhouse	2	Do.
		do	Field	3	Do.
	7427	Seedling	Greenhouse	1	Do.
		do	Field	5	Do.
	7775E	Cutting	do	2	Do.

<sup>a</sup> Crop Physiology and Breeding Investigations.<sup>b</sup> On *Aeglopsis chevalieri*.<sup>c</sup> On *Aegle marmelos*.

TABLE II.—Relative susceptibility of citrus fruits to canker

Genus and species	C. P. B. No. <sup>a</sup>	Type of plants	Location	Num-ber of plants	Results	Remarks
<i>C. hystrix</i> , pointed-leaf form	7727 7872	Seedling do	Field Greenhouse	2 2	Susceptible minus do	Leaves and occasional twig. Do.
<i>C. hystrix</i> , round-leaf form	2831 7831	do do	Field Greenhouse	12 1	Susceptible plus do	Leaves, twigs, and round wood. Do.
<i>C. medica</i> , Citron of Commerce	7768 7836 7816 11294 11281 11178	Cutting Seedling do do do do	Field Greenhouse do do do do	2 2 2 2 2 2	Susceptible do do do do do do	Do. Do. Do. Do. Do. Do.
<i>Citrus</i> sp. small lemon	7833	do	do	2	do	Do.
Sweet	1158	do	do	2	do	Do.
"Davao"	7837	do	do	2	do	Do.
"Limon real" No. 18	7819	do	do	2	do	Do.
Ichang	11204-A	do	do	2	do	Do.
<i>C. limonia</i> , large lemon	11291	do	do	3	do	Leaves and occasional twig. Leaves, twigs, and occasionally round wood.
<i>C. aurantifolia</i> , sour lime	7832	do	Field	4	do	Leaves and occasional twig.
<i>C. grandis</i> , grapefruit	7338 7903 11170	do do do	do do Greenhouse	2 2 6	do do Susceptible plus	Leaves, twigs, and round wood. Leaves and occasional twig. Leaves, twigs, and round wood.
Sullivan	Alabama	do	do	5	do	Do.
	11001	do	Field	3	do	Do.
	11054	do	do	3	do	Do.
Shaddock Florida	11255	do	do	3	do	Leaves, twigs, and round wood.
Pummelo	7834	do	Greenhouse	2	do	Do.
Hirado Buntan	7993	do	do	3	Susceptible minus	Leaves and occasional twig.
Indian	2968-A	do	Field	3	do	Do.
	11163	do	Greenhouse	2	Susceptible plus	Leaves, twigs, and round wood.
	11168	do	Field	2	do	Do.
Siamese	6111	Budded <sup>b</sup>	do	2	do	Do.
	11201	Seedling	Greenhouse	1	Susceptible minus	Leaves only.
Chinese Orangedale	11212U	do	Field	1	Susceptible	Do.
French Martin	11213S	do	Greenhouse	2	do	Do.
Mark's	11061	do	do	3	Susceptible minus	Leaves and occasional twig.
	11217F	do	Greenhouse	1	Susceptible plus	Leaves, twigs, and round wood.
	11217G	do	Field	2	do	Do.
		do	do	3	Susceptible	Leaves, twigs, and occasionally round wood.
		do	do	3	do	Do.

<sup>b</sup> On *Citropsis schweinfurthii*.<sup>a</sup> Crop Physiology and Breeding Investigations.

TABLE II.—Relative susceptibility of citrus fruits to canker—Continued

Genus and species	C. P. B. No.	Type of plants	Location	Num-ber of plants	Results	Remarks
C. sinensis, orange	66A	Seedling	Greenhouse	2	Susceptible	Leaves and occasional twig.
	7773	do.	do.	3	do.	Do.
	11324	do.	do.	2	do.	Do.
	45344	Budded*	Field	1	do.	Do.
	11164	Seedling	do.	2	do.	Do.
	do.	do.	do.	3	do.	Leaves, twigs, and occasionally round wood.
	11198	do.	do.	3	do.	Do.
	11199	do.	do.	3	do.	Leaves and occasional twig.
	11023	do.	do.	3	do.	Do.
	7560A	do.	do.	6	do.	Do.
C. nobilis, King of Siam	7562	do.	do.	2	Susceptible plus	Leaves, twigs, and round wood.
	11028	do.	do.	6	Susceptible	Leaves and occasional twig.
	11313	do.	do.	2	Susceptible minus	Do.
	2105	do.	Greenhouse	6	do.	Do.
	11384	do.	Field	2	do.	Do.
	7830	do.	Greenhouse	2	do.	Do.
	Alabama	Budded*	do.	2	do.	Leaves only.
	11195	Seedling	do.	3	do.	Do.
	11338	do.	do.	2	do.	Do.
	7065	do.	Field	18	do.	Leaves and occasional twig.
C. mitis, Calamondin	11265	do.	Greenho	3	do.	Do.
	do.	do.	do.	2	do.	Do.
	11312	do.	Field	4	do.	Leaves only.
	do.	do.	do.	17	do.	Do.
	44305	do.	Greenhouse	2	do.	Leaves and occasional twig.
	7908	do.	Field	3	do.	Leaves only.
	11242	do.	Greenhouse	1	do.	Do.
	11298	do.	Field	3	do.	Leaves and occasional twig.
	do.	Budded*	do.	1	do.	Do.
	do.	Budded*	do.	1	do.	Do.
Citrus sp. Natsu-mikan	11184	Seedling	Greenhouse	3	Susceptible	Leaves, twigs, and occasionally round wood.
	do.	do.	Field	3	do.	Do.
	11197	do.	do.	4	do.	Do.
	11337	do.	do.	3	do.	Do.
	7827	do.	Greenhouse	2	Susceptible plus	Leaves, twigs, and round wood.
	7820	do.	do.	2	do.	Do.
	7818	do.	do.	1	Susceptible minus	Leaves and occasional twig.
	7829	do.	do.	3	Susceptible	Do.
	do.	do.	Field	2	do.	Leaves, twigs, and occasionally round wood.
	11196	do.	do.	3	do.	Do.
C. excelsa	11280	do.	Greenhouse	2	do.	Do.

\* On *Poncirus trifoliata*.

\* On *Citrus grandis*.

\* On *Serentinia buxifolia*.

TABLE III.—Relative susceptibility of citrus hybrids to canker

Hybrid	C. P. B. <sup>a</sup> No.	Type of plant	Location	Num- ber of plants	Results	Remarks
Faustime (M. australasica × C. aurantifolia)	49819	Cutting	Greenhouse	1	Susceptible	Leaves, twigs, and round wood.
	49823	do	do	1	do	Do.
	49835	do	do	1	do	Do.
	49841	do	do	1	do	Do.
	49843	do	do	1	do	Do.
Faustrimon (M. australasica × C. limonia)	49843	do	do	1	do	Do.
	49844	do	do	1	do	Do.
	47431	do	do	2	do	Leaves and occasional twig.
		do	Field	2	do	Do.
	772A C	Seedling	do	2	Susceptible plus	Leaves, twigs, and round wood.
Citrange Colman (P. trifoliata × C. sinensis)	7896	do	Greenhouse	2	do	Do.
	1418A A	do	Field	2	do	Do.
	7965	do	Greenhouse	2	do	Do.
	761A C	do	do	2	do	Do.
	771A	do	do	2	do	Do.
Morton	716	do	Field	2	do	Do.
	7895	do	do	10	do	Do.
	7895 Y	do	Greenhouse	2	do	Do.
	7956	do	do	2	do	Do.
		do	Field	5	do	Do.
Rusk	11030	do	Greenhouse	3	do	Do.
		do	do	2	do	Do.
	44980	do	Field	1	do	Do.
		do	do	1	do	Do.
	777A B	do	Greenhouse	3	Susceptible	Leaves and occasional twig.
Willits	7897	do	do	2	do	Do.
	7960	do	do	2	do	Do.
	779A A	do	Field	3	do	Do.
	7898	do	do	4	do	Leaves, twigs, and round wood.
	7961	do	Greenhouse	2	do	Do.
Savage		do	Field	5	do	Do.
		do	do	3	do	Do.
	1423A B	do	do	2	do	Do.
	7963	do	Greenhouse	2	do	Do.
	7934 A	do	do	2	do	Do.
Etonia	749A B	do	Field	2	do	Do.
	1416	do	Greenhouse	2	do	Do.
	1425A B	do	Field	1	do	Do.
	43434	do	Greenhouse	2	do	Do.
	43480	do	do	2	do	Do.
Citrange	43931	do	do	2	do	Do.
	1425A A	do	Field	2	do	Do.
	4475A	do	Greenhouse	1	do	Do.
	4493	do	do	2	do	Do.
	4554	do	do	2	do	Do.
Citrumelo (P. trifoliata × C. grandis)	4564	do	do	4	do	Do.
		do	do	1	do	Do.
		do	do	2	Susceptible	Leaves, twigs, and occasionally round wood.
	50850	do	do	2	do	Do.
		do	do	2	do	Do.

<sup>a</sup> Crop Physiology and Breeding Investigations.



TABLE III.—Relative susceptibility of citrus hybrids to canker—Continued

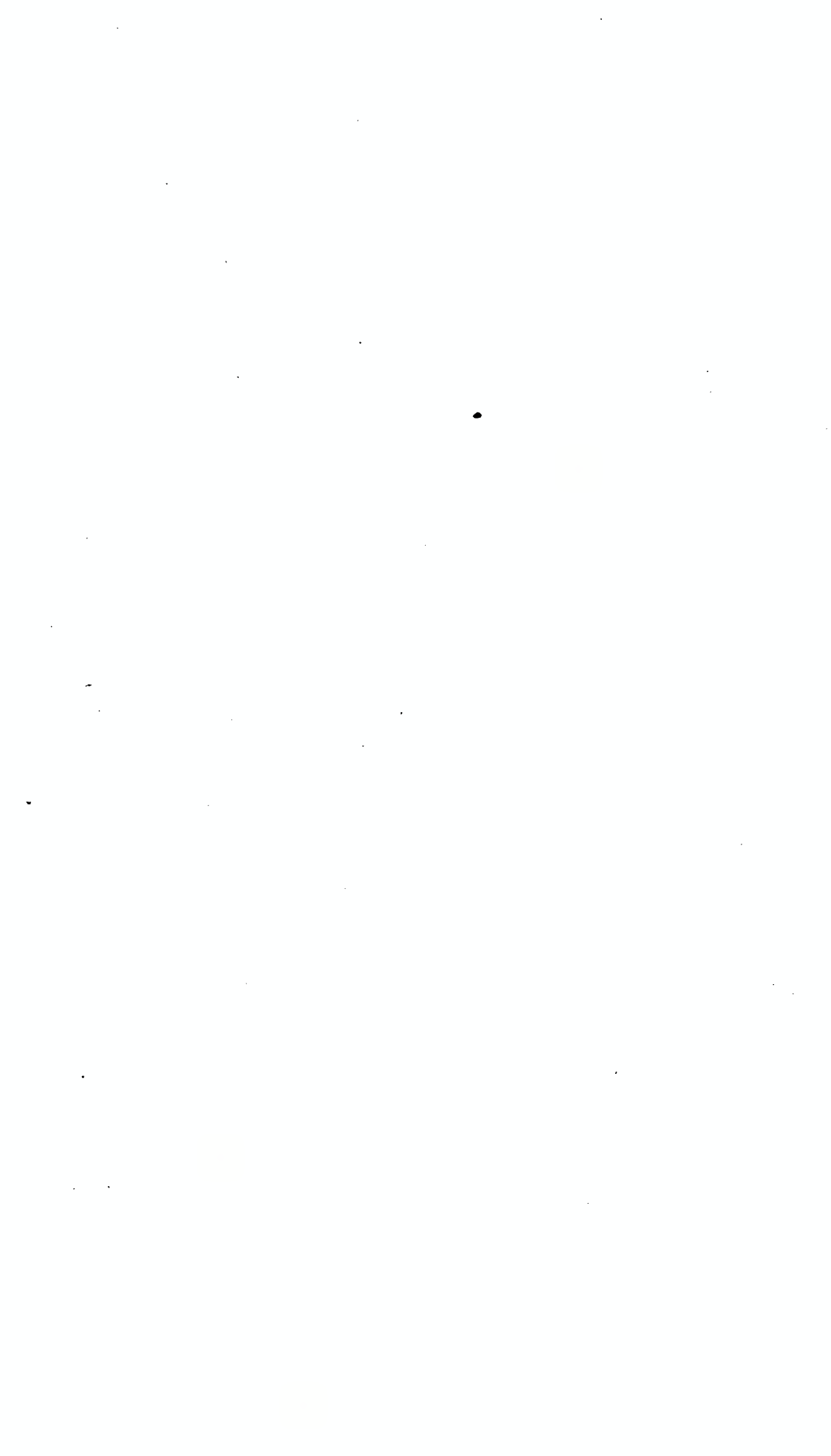
Hybrid	C. P. B. No.	Type of plant	Location	Num-ber of plants	Results	Remarks
Citrandarin (P. trifoliata×C. nobilis)	40175A	Seedling	Field	4	Susceptible	Leaves, twigs, and occasionally round wood.
	40210	do	Greenhouse	3	do	Do.
	40303C	do	do	2	do	Do.
	40315	do	do	2	do	Do.
	40368B	do	do	2	do	Do.
	48529	Budded <sup>b</sup>	do	2	do	Do.
	48720	Seedling	Field	1	do	Do.
	48721	do	do	1	do	Do.
	48724	do	do	1	do	Do.
	48726	do	do	1	do	Do.
Citrandarin (P. trifoliata×C. nobilis unshiu)	48732	do	do	1	do	Do.
	48737	do	do	1	do	Do.
	48746	do	do	1	do	Do.
	48748	do	do	1	do	Do.
	48824	do	do	1	do	Do.
	48825	do	do	1	do	Do.
	48844	do	do	1	do	Do.
	48863	do	do	1	do	Do.
	48864	do	do	1	do	Do.
	48866	do	do	1	do	Do.
Citrandarin (P. trifoliata×C. nobilis deliciosa)	48888	do	do	1	do	Do.
	48895	do	do	1	do	Do.
	48899	do	do	1	do	Do.
	48712	do	do	1	do	Do.
	48607	do	do	1	do	Do.
	48608	do	do	1	do	Do.
	48611	do	do	1	do	Do.
	48615	do	do	1	do	Do.
	48620	do	do	1	do	Do.
	48623	do	do	1	do	Do.
Citrunshu (P. trifoliata×C. nobilis unshiu)	51102	Budded <sup>b</sup>	do	1	do	Do.
	48290	Seedling	Greenhouse	2	Susceptible plus	Leaves, twigs, and round wood.
	48316	do	do	3	do	Do.
	48003	Budded <sup>c</sup>	Field	1	No infection	Leaves only.
	48005	Budded <sup>d</sup>	do	1	Very resistant	Do.
	48007	Budded <sup>e</sup>	do	1	No infection	Do.
	48010	Seedling	Greenhouse	2	Very resistant	Do.
	48010D2	Budded <sup>c</sup>	Field	1	No infection	Do.
	48010D5	Seedling	Greenhouse	3	Very resistant	Do.
	48010E1	Seedling	Field	2	do	Do.
Citrangequat (Willits citrange x F. margarita)	48010E	do	do	4	do	Do.
	48010E	do	do	1	No infection	Do.
	48010E	Budded <sup>f</sup>	do	1	do	Do.
	48010F	Seedling	do	4	Leaves only	Very resistant.
	48011	Budded <sup>d</sup>	do	1	No infection	Do.



TABLE III.—Relative susceptibility of citrus hybrids to canker—Continued

Hybrid	C. P. B. No.	Type of plant	Location	Num-ber of plants	Results *	Remarks
Orangequat (C. sinensis×F. margarita)	49056	Budded <sup>t</sup>	Field	1	Susceptible	Leaves only.
	50312	Budded <sup>b</sup>	do	1	do	Leaves and occasional twig.
	48812	Budded <sup>s</sup>	do	1	do	Do.
	50304	Budded <sup>d</sup>	Greenhouse	1	do	Leaves only.
		Budded <sup>s</sup>	Field	1	do	Leaves and occasional twig.
	52001R1	do	do	1	do	Do.
	52003I2	do	do	1	do	Do.
	52009A2	Budded <sup>d</sup>	Greenhouse	1	do	Leaves only.
	52009G5	do	do	1	do	Leaves, twigs, and round wood.
	52009Q1	Budded <sup>s</sup>	Field	1	do	Leaves and occasional twig.
Satsumelo (C. nobilis unshiu×C. grandis)	52009V4	do	do	1	do	Do.
	52010F1	do	do	1	do	Do.
	52011M1	do	do	1	do	Do.
	52011R1	Budded <sup>d</sup>	do	1	do	Leaves only.
	52011Y4	do	do	1	do	Leaves, twigs, and round wood.
	52022P1	Budded <sup>s</sup>	do	1	do	Leaves and occasional twig.
	52032H1	do	do	1	do	Do.
	52304	Budded <sup>b</sup>	do	1	do	Do.
	49006	Seedling	Greenhouse	1	Susceptible plus	Leaves, twigs, and round wood.
	49013	Budded <sup>b</sup>	do	1	do	Do.
Clemelo (C. nobilis deliciosa×C. grandis)	49025	Budded <sup>b</sup>	do	1	do	Leaves and twigs.
		Budded <sup>d</sup>	do	1	do	Do.
		Budded <sup>b</sup>	do	1	do	Do.
	49028	Budded <sup>b</sup>	do	1	do	Do.
	49032	do	do	1	do	Do.
	49038	do	do	1	do	Do.
	49043	Budded <sup>d</sup>	do	1	do	Leaves, twigs, and round wood.
	49049	Budded <sup>b</sup>	do	1	do	Leaves and twigs.
	47355B	Seedling	Greenhouse	1	do	Leaves, twigs, and round wood.
	50520	Budded <sup>d</sup>	Field	2	Susceptible	Leaves and twigs.
Siamelo (C. nobilis×C. grandis)	50535	do	do	1	do	Leaves and twigs.
		do	do	1	Susceptible plus	Do.
	50551	do	do	1	Susceptible	Leaves and twigs.
	51531	do	do	1	do	Do.
	51565	do	do	1	do	Do.
	51584	do	do	1	do	Do.
	51586	do	do	1	do	Do.
	51598	Seedling	Greenhouse	1	do	Do.
	51947	do	do	1	do	Do.
	51969	Budded <sup>d</sup>	Field	1	do	Do.
52007-O6	52007-O6	do	do	1	do	Do.
	52007-P3	do	do	1	do	Do.
	52007-P11	do	do	1	do	Do.

[illegible]



# RELATION OF ENVIRONMENTAL FACTORS TO CITRUS SCAB CAUSED BY CLADOSPORIUM CITRI MASSEE<sup>1</sup>

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## INTRODUCTION

In connection with citrus canker investigations conducted by the writers, an opportunity was afforded to observe the behavior of citrus scab caused by *Cladosporium citri* Massee under the weather conditions prevailing in southern Alabama for four seasons. In the following pages an attempt is made to correlate field observations with studies made under controlled conditions in the laboratory and greenhouse on the pathogene, on the host plant, and on infection and development of the disease.

## HISTORY AND DISTRIBUTION

Scab attacks the young fruit, leaves, and occasionally the shoots of Citrus plants. The effect of scab on the fruits is the most important aspect of the disease from an economic viewpoint, in that scabby fruit either results in lower market value or in cull fruits.

What probably constitutes the earliest record of scab was found by Lee (5)<sup>2</sup> on a herbarium specimen of *Citrus nobilis* Lour. collected at Nagasaki, Japan, in 1863. He states that the early occurrence of this disease in Japan and the general distribution of citrus scab throughout the Citrus-growing regions of South China indicate that this disease may be indigenous to the Orient.

Reinking (7, 8), in a plant-disease survey of southern China found scab generally prevalent in this region, which substantiates Lee's conclusions. Considerable work has been done on scab in Japan, as it is there one of the more important diseases of Citrus, but owing to the inaccessibility of the Japanese literature it is difficult to obtain much information regarding the history of scab in that country. No doubt, as in the case of the citrus canker, Japan has indirectly served as the center for the distribution of scab to some of the countries in which the disease is now present. For several decades large exportation of Citrus nursery stock to various parts of the world has been made from several Japanese ports. Thus it is possible that Japan served as the center for scab distribution to Australia, the Gulf Coast section of the United States, and South Africa, as it did in the case of canker. So far as is known, no scab has ever been reported from California and the Philippine Islands, although there is every reason

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WINSTON, J. R.

1923. CITRUS SCAB: ITS CAUSE AND CONTROL. U. S. Dept. Agr. Bul. 1118, 38 p., illus.

TANAKA, T.

1923. A BRIEF HISTORY OF CITRUS SCAB IN JAPAN. *Phytopathology* 13:492-495.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 254.

to assume that scab has been introduced into these countries on nursery stock. Further, Reinking (8) does not mention the presence of scab in the Citrus-growing regions of Siam and Indo-China.

Fawcett (2) summarized the California situation as follows: "This disease (scab) has never been found in California. Before the strict quarantine laws were passed, thousands of sour orange trees with their leaves affected with scab were brought into California, but the new foliage came out free from attack. It would appear that this fungus is unable to persist in a climate like that of California."

According to Swingle and Webber (9), scab appeared in Florida about 1884 and spread rapidly over the State and into Louisiana. They state that it was probably introduced into America from Japan. Since that time it has been introduced from Florida and Louisiana, or direct from Japan on the many importations of nursery stock, especially Satsuma (*Citrus nobilis unshiu* (Makino) Swingle, into the Gulf Coast States of Alabama, Mississippi, and Texas. Thus, at the present time, scab is one of the more important diseases of Citrus fruits in the Gulf Coast States, although fortunately it can be controlled by spraying. Scab has also been reported from India, Formosa, the West Indies, Paraguay, the Canal Zone, Yucatan, and Hawaii.

Scab is a rather serious disease in the Gulf Coast States, South China, Japan, and parts of the West Indies. So far it has not been reported from the Citrus-growing regions of the Mediterranean countries, California, and the Philippine Islands. The absence of scab in these localities is an interesting phase of the scab situation. That scab has been introduced on nursery stock into California and the Philippine Islands, and possibly the Mediterranean countries without gaining a foothold, indicates that there are certain factors which prevent the successful propagation of citrus scab in these localities.

## INFLUENCE OF ENVIRONMENTAL FACTORS UNDER CONTROLLED CONDITIONS

### ON THE PATHOGENE

Fawcett (1, 2) has continually emphasized the fact that *Cladosporium citri* differs from other species of *Cladosporium*, and especially *C. herbarum* Lk., which is usually associated with *C. citri* on old scab spots. In fact, *C. herbarum* has in some cases been mistaken for the causal organism. He has so well described the difference in appearance of these organisms both in pure culture and on the plants themselves, that it will be sufficient to state here that *C. citri* is an unusual *Cladosporium*, which makes an extremely small and slow growth in pure culture.

The optimum temperature, as worked out by Fawcett (4) for the best development of the fungus in pure culture, is 21° C. (69.8° F.). Growth occurs in culture within a range of temperatures between 13.5° and 32° C. (56° and 89.6° F.). Judging from Fawcett's results, spore production in pure cultures seems to be limited to temperatures between 16° and 27.5° C. (60.8° and 81.5° F.).

It has also been observed by Fawcett (1) that the spores of this fungus germinate readily both in tap water and on agar; germination of the spores beginning in from 5 to 24 hours. It is not known whether the spores will germinate in a saturated atmosphere or at lower humidities.

### ON THE HOST PLANTS

The results obtained by the senior writer (6) at the University of Illinois indicate that each type of Citrus plant reacts differently under similar environmental conditions. Under controlled conditions, grapefruit plants (*Citrus grandis*

(L.) Osbeck) made an extremely slow growth at 18° C. (59° F.), while the other plants tested did not start until a temperature of 20° C. (68° F.), was reached. Even at this temperature *C. mitis* Blanco, a native of the Philippine Islands only made a slow growth. At 30° C. (86° F.) all plants tested developed rapidly. Above this point the growth of plants of the grapefruit type were inhibited, while *Poncirus trifoliata* (L.) Raf. made a good growth.

It was also found that at 15° C. (59° F.), grapefruit plants, although making an extremely slow growth matured their foliage rapidly, in most instances within a week's time, while at 30° C. (86° F.) growth was rapid, but the period of leaf maturation was extended over a period of about two weeks. The mature leaves at the low temperature were only from one-fourth to one-half the size of those on plants held at 30° C. (86° F.). The small leaves maturing at the lower temperature retained their same size when transferred to higher temperatures.

Plants held at 30° C. (86° F.) were inhibited in their growth when transferred to lower temperatures. The growth of citrus plants was inhibited when held at a high temperature during the day and at a low temperature during the night.

#### ON INFECTION AND DEVELOPMENT OF THE DISEASE

As far as the writers are aware, no one has successfully infected Citrus plants with *Cladosporium citri* under field conditions. Even in the greenhouse, infection has not always been successful. In practically all cases the only successful infections which have been produced under greenhouse conditions have been made on plants held under bell jars, or where abundant moisture on the surface of the leaves was supplied for a period of several days. In this connection it should also be stated that only the young leaves have been infected.

In the experiments reported by Fawcett (4), and in the observations made by the senior writer, the two factors of young growth and sufficient moisture for initial infection have always been supplied. Fawcett inoculated his plants and placed them in the temperature chambers, with sufficient moisture, for a period long enough for initial infection to take place. In the senior writer's observations on scab, the plants were held in temperature cases in a saturated atmosphere under bell jars.

Fawcett (4) has shown that the temperature limits for successful infection of sour orange and pummelo plants under controlled conditions lies between 16° and 23° C. (60.8° and 74.3° F.). No infections were obtained at 14° (57.2° F.) or below, nor at 24.5° C. (76.1° F.) or above, so that infection is limited to a rather narrow range of temperature. The largest number of scab spots occurred at the temperatures between 18.5° and 21° C. (65.3° and 69.8° F.).

In the senior writer's experiment with citrus canker (6) optimum conditions for scab development were afforded and typical scab spots did occur naturally on some of the plants growing in the temperature cases at 15° and 20° C. (59° and 68° F.). No scab appeared on plants held at temperatures of 10° C. (51° F.) or below, nor at 25° C. (77° F.) or above. At 15° C. (59° F.) scab spots occurred only on the grapefruit plants showing new growth. It should be noted that grapefruit were the only plants making any growth at this temperature. At 20° C. (68° F.) scab appeared on grapefruit, calamondin, and citrange plants. The spots were much more numerous on grapefruit than at 15° C. (59° F.). In all cases, scab was limited to the plants with young leaves.

The results show that when abundant moisture is supplied and young growth is present, infection of Citrus plants by *Cladosporium citri* is limited to temperatures between 15° C. and 23.5° C. (59° and 74.3° F.), a range of less than 10 degrees. The optimum for the best and greatest development of scab appeared to be about 20°–21° C. (68°–69.8° F.), which is also the optimum found by Fawcett (4) for the fungus in culture.



## INFLUENCE OF ENVIRONMENTAL FACTORS UNDER FIELD CONDITIONS

## ON THE HOST PLANTS

Citrus scab is usually prevalent during the development of the first spring growth. Under controlled conditions the maximum temperature for successful infection is about 75° F. Under Alabama conditions a mean weekly temperature of 75° F. and above is reached and the first spring growth of the plants is usually completed by the first of June. For the purpose of our discussion, then, we are interested primarily in the behavior of the plants during the interval required for the development of the first growth period.

It might be of interest in this connection to correlate the appearance of new growth of the more common Citrus plants under Alabama conditions, with the prevailing temperatures recorded at Mobile for the first five months of the years 1914 to 1920.

In Table I are listed the weekly mean temperatures from January 1 through June 10 for these years. In figure 1 the weekly mean temperatures for 1918 and 1916 are plotted with the two late seasons of 1915 and 1920 for comparison.

In 1915 no weekly mean temperatures suitable for active growth of Citrus plants were recorded until the week of April 9-15, when the mean rose to 67° F. and proceeded rapidly upward. This temperature was sufficient to force all plants into activity at practically the same time.

During the early part of 1916 the weekly mean temperature rose to 59° F. and above on three separate occasions, each of which was sufficient to force active growth of grapefruit plants but not Satsuma or trifoliolate orange. In each case, however, these temperatures were followed by killing frosts. It was not until the week of March 19-25 that the weekly mean temperature was high enough to force new growth. The mean for this week was 68° F. which was high enough to force all Citrus plants into active growth.

While January, 1918, was conspicuous for its low temperatures, weekly mean temperatures for forcing and continuing growth of grapefruit plants occurred from the middle of February on. By the last of February the weekly mean temperatures were high enough for Satsuma and trifoliolate orange to develop. Notes on the plants made in the isolation field on March 8 showed that the majority, which survived the low temperatures of January, were in full leaf or starting growth. In fact, scab was observed on a number of plants on this date. Without any question, the season of 1918 was the earliest under discussion.

During the 1920 season temperatures high enough to cause active growth of grapefruit plants and swelling of the trifoliolate orange and Satsuma buds occurred during periods in January and February. In fact, grapefruit plants were almost in full leaf when killed back by the freezes of February 15 and 16. The lowest temperatures of the season occurred during the first week in March. All new growth which occurred prior to that time was killed back. With the advent of higher weekly mean temperatures it was some time before new growth started. The late season of 1920, then, was due to the destructive frosts the first week in March. The development of the uninjured buds was also delayed until suitable temperatures were again at hand. To summarize, most of the plants were in full leaf or were starting growth the week ending February 4 in 1918, March 25 in 1916, April 1 in 1920, and April 15 in 1915.

From the above notes it can be clearly seen that the dormancy of Citrus plants during the early part of the year is variable and depends to a large extent on the prevailing temperatures. The seasons of 1914, 1915, and 1920 can be

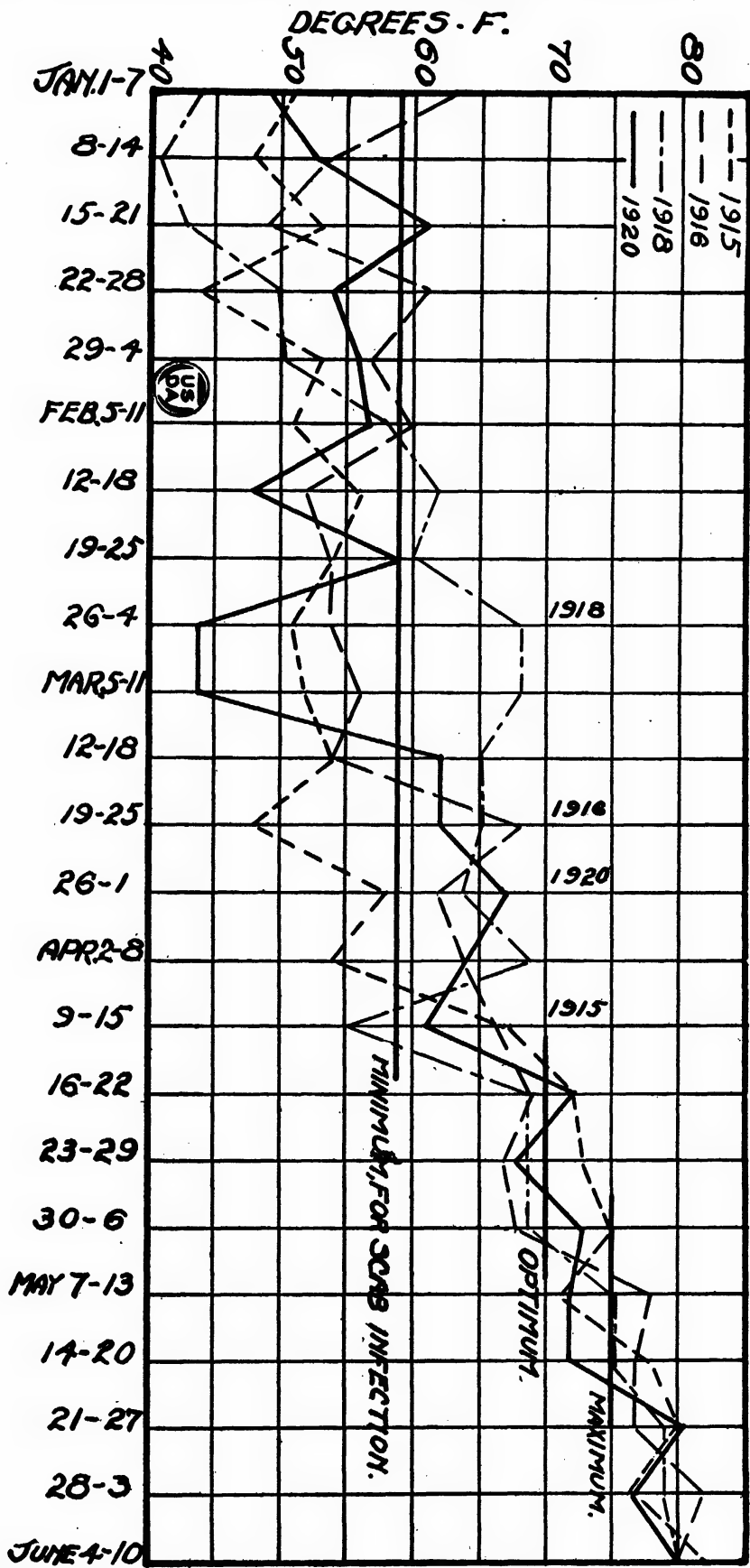


FIG. 1.—Graph showing the weekly mean temperature from January 1 to June 10, for the years 1915, 1916, 1918, and 1920 at Mobile, Ala., together with the minimum, optimum, and maximum temperatures for citrus-scab infection

classed as late, the season of 1918 as extremely early, while the remainder were more or less normal.

When the temperature is correlated with the appearance of new growth in Alabama, grapefruit plants are forced into new growth at any time after the first of the year when a weekly mean temperature of 59° F. or above prevails. For the trifoliate orange and Satsuma, a weekly mean temperature of above 65° F. is necessary.

Not all plants of the same variety start growth at the same time in the spring. Thus, we not only have various species starting at different times, but even plants of the same variety. It is also a well-known fact that the first spring growth is usually smaller than that produced during the succeeding growth periods. The leaves formed are also smaller and mature more rapidly. Thus the amount of spring growth formed and the rapidity with which it matures are also dependent on the prevailing weather conditions.

TABLE I.—Weekly mean temperatures from January 1 through June 10, for 7 years at Mobile, Ala.

Month	1914	1915	1916	1917	1918	1919	1920
	° F.	° F.	° F.	° F.	° F.	° F.	° F.
Jan. 1-7	44.0	51.0	63.0	61.0	44.0	40.0	49.0
8-14	51.0	48.0	54.0	53.0	41.0	47.0	53.0
15-21	60.0	53.0	49.0	53.0	43.0	53.0	61.0
22-28	58.0	44.0	61.0	57.0	50.0	57.0	54.0
29-Feb. 4	56.0	53.0	57.0	50.0	50.0	55.0	56.0
Feb. 5-11	53.0	51.0	60.0	45.0	58.0	49.0	57.0
12-18	52.0	56.0	52.0	56.0	62.0	53.0	48.0
19-25	51.0	54.0	54.0	58.0	60.0	59.0	59.0
26-Mar. 4	48.0	51.0	54.0	68.0	68.0	56.0	44.0
Mar. 5-11	53.0	52.0	56.0	56.0	68.0	58.0	44.0
12-18	54.0	54.0	54.0	67.0	65.0	65.0	62.0
19-25	50.0	48.0	68.0	63.0	65.0	65.0	62.0
26-Apr. 1	68.0	58.0	62.0	65.0	64.0	59.0	67.0
Apr. 2-8	67.0	54.0	64.0	62.0	69.0	63.0	64.0
9-15	60.0	67.0	59.0	61.0	55.0	69.0	61.0
16-22	68.0	72.0	69.0	69.0	69.0	64.0	72.0
23-29	74.0	73.0	67.0	73.0	69.0	70.0	68.0
30-May 6	76.0	75.0	68.0	69.0	69.0	72.0	73.0
May 7-13	73.0	71.0	78.0	63.0	75.0	73.0	72.0
14-20	72.0	78.0	77.0	69.0	75.0	73.0	72.0
21-27	75.0	80.0	77.0	77.0	79.0	68.0	80.0
28-June 3	80.0	77.0	82.0	79.0	79.0	75.0	77.0
June 3-10	83.0	82.0	80.0	80.0	80.0	81.0	77.0

## ON THE DISEASE

Judging from the results obtained under controlled conditions, we would expect initial infection to occur in the field on young growth during periods when free moisture was present on the surface of the leaves between temperatures of 59 and 74.3° F. Initial infection would be most abundant under these conditions when a temperature of about 68 to 70° F. prevailed.

Observations point to the fact that the spores of the pathogene overwinter on the leaves in the old scab spots formed during the preceding season. At least these old scab spots serve as the chief source for the early spring infections. Judging from the many observations made in the field, it appears that the spores of the fungus may also lodge in the scales of the dormant buds and then when these begin to grow in the spring the spores are at hand to produce infection on the young unfolding leaves.

The presence of free moisture on the surface of the leaves is necessary for a rather long interval for the successful germination of the spores to take place and for the germ tube to enter the tissues of the leaf. Even after the hyphae

have entered the tissues, the small and slow amount of growth which the fungus makes necessarily lengthens the period of incubation. The period of initial infection for citrus scab is a question of hours and not minutes. A summary of Fawcett's (4) infection experiments shows that the period of incubation is also long, six days to two weeks being required even under optimum conditions for infection.

Primary infection in the spring usually occurs on the unfolding buds, while secondary infection takes place on the young fruits very soon after the petals drop. It is rather unusual under Alabama conditions for the pathogene to attack half-grown leaves or fruits. It is true that scab is found on leaves and fruits of this age, but the length of the incubation period indicates that initial infection takes place while the leaves and fruits are quite small.

The fact that the disease appears on either side of the leaves, precludes the possibility of stomatal infection as no stomata are present on the upper surfaces of Citrus plants. Infection is accomplished, for the most part, by the direct penetration of the epidermis by the fungus hyphae. As the leaves mature the firmer leaf texture would necessarily become less penetrable by the hyphae of the fungus.

Initial infection is not dependent on rapidly growing tissue so much as on the fact that the leaves must be young, whether rapidly growing or not. However, after initial infection takes place, rapidly growing infected tissues produce more malformations and consequently larger and more misshapen leaves and fruits. Any conditions favoring the rankness of the first growth would also contribute to produce larger scab spots.

In figure 1, the minimum, optimum, and maximum temperatures for scab infection, as determined under controlled conditions, are indicated. While these points may not be strictly applicable to what actually occurs in the field, they are sufficiently accurate for the purpose of our discussion. During the early part of some years, the weekly mean temperatures rise above the minimum for infection for short periods, but in most instances, they are followed by sudden drops in temperature. No scab has ever been found during these periods, as the other two conditions for successful infection are not always at hand; namely, sufficient moisture and the presence of young foliage. Temperatures suitable for slight infection occur at different times during the various seasons, as indicated in figure 1. The period during which optimum temperatures for infection prevail is usually from the middle of April through most of May. By the third week in April the temperature during most years reaches the optimum and by the first of June the prevailing temperatures are above the maximum for infection.

If the temperature factor is considered by itself, we should expect the same amount of scab each year, as the optimum temperature for infection is reached at practically the same time. There is only one week or at the most, two weeks' difference in the time required for the temperatures to reach the maximum. We should also expect that the earlier the season the more prevalent scab would be. However, it is exactly the reverse, as will be brought out later.

The presence of moisture over a long enough period for initial infection to take place has been continually stressed as one of the essentials for successful infection. The weekly rainfall, including the number of rainy days per week for the spring season of 1914 to 1920, was tabulated, but no positive correlations between the relative prevalence of scab and the amount or frequency of rainfall could be made. Under Alabama conditions, there is rarely a week in which rain does not occur during the development of the first spring growth. In the majority of cases, when temperatures suitable for infection are at hand, moisture sufficient for initial infection is present. Thus, as far as Alabama is concerned, we can take

it for granted that the rainfall is sufficient during the greater part of the time in which temperatures suitable for infection prevail.

We come now to the most important variable factor and by all means the most difficult to present, namely, the development of the early spring growth and its relation to scab.

In the preceding pages, it has been pointed out that the various types of Citrus plants start growth in the spring when different weekly mean temperatures are reached. These points vary during different years so that in some cases they may occur as much as six weeks apart. Again, not all plants of the same variety start at the same time. Furthermore, during certain seasons all plants may start at practically the same time, or at different times, during years when the weekly mean temperatures are low. The amount of first spring growth and the rapidity with which it matures are also dependent on weather conditions. Thus, all these points must be taken into consideration in discussing the relative prevalence and susceptibility to scab of various plants.

Any environmental factor or factors which induce a slow or slight spring growth and rapid maturation or late starting of susceptible Citrus trees favor scab escape; while any environmental factor or factors which induce a large amount of spring growth and subsequently slower maturation, especially during the period of optimum infection, favor scab attacks. Thus, we must not only consider the internal factors influencing the development of spring growth of the Citrus trees, but also the external factors which may inhibit or stimulate these processes.

To illustrate, grapefruit plants, as a rule, start rather early in the season and during normal years complete their growth before optimum conditions favorable for scab are at hand. However, plants may go into the winter in a devitalized condition and so start extremely late in the spring and thus escape infection. It is only when environmental conditions are such that the development of the first spring growth coincides with the optimum conditions for scab development that the disease is at all serious on grapefruit plants.

As is well known, Satsumas are generally susceptible to scab. The somewhat higher temperature necessary for the forcing of active growth of Satsuma usually means that it starts later in the season and first spring growth is well developed at the time optimum conditions for infection are at hand.

Until more data are at hand, we can roughly divide the susceptible commercial Citrus species and varieties into three groups, according to their normal development (internal) in the field as follows:

1. Varieties which start early in the season and make a slow and slight growth, which matures rapidly.
2. Varieties which start somewhat later, and produce a larger amount of spring growth, which matures slowly and about the time optimum conditions for infection are at hand.
3. Varieties which start late in the season after the optimum for infection has occurred.

Plants falling in Groups 1 and 3 are generally free from scab, while those in Group 2 are usually easily infected and badly attacked by scab.

External factors, however, play an equally important part in determining the type of spring growth developed and in its scab susceptibility or escape. After a thorough consideration of the numerous factors involved, it is not surprising that no successful infections have been made in the field with this disease. However, it should not be difficult to do when all factors are taken into consideration. Evidently some essential requirements for successful infection in the experiments so far reported have been omitted.

CONDITIONS DETERMINING THE YEARLY PREVALENCE OF CITRUS SCAB

It is a well-known fact that there are epidemic and nonepidemic scab seasons. Thus, in Alabama, the seasons of 1914, 1915, and 1920 were noted as very bad scab years. The season of 1918 was probably the lightest scab year recorded and yet scab was plentiful on some plants in the isolation field as early as March 8. Some scab was present during the years 1916, 1917, and 1919, but these can be classed as nonepidemic years.

Although scab is present during the month of March in some seasons, the weather conditions prevailing during April and May determine largely the relative prevalence of scab. To determine the conditions necessary for an epidemic or nonepidemic year, the seasons of 1916 and 1918 are contrasted with those of 1915 and 1920 (fig. 1).

To facilitate the discussion, it can be said that most plants were in full leaf or were starting growth by the week ending March 4, during 1918. In other words, the mean temperatures for February and March were above the normal for these months, so growth responded very early in the season. Note also that the prevailing temperatures did not approach the optimum for infection until well along in April, by which time the plants had about completed their first spring growth, and so escaped the disease. Let us contrast these conditions with those of 1915. The monthly mean temperature for March was almost 7° below the normal. No temperatures suitable for growth and subsequent infection occurred until the middle of April. The following week, just as the young growth was developing the weekly mean temperature went above the temperature for optimum infection and remained more or less at this point for several weeks. During the first two weeks in May almost 4 inches of rainfall occurred on eight different days. The three conditions essential for infection were thus well supplied so that the year 1915 is noted as the worst scab year on record in Alabama.

TABLE II.—Monthly mean temperature, precipitation, and number of rainy days at various localities

MONTHLY MEAN TEMPERATURE													
Station	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	An-nual
Manila, P. I.....	77.0	77.2	80.2	82.9	83.5	82.2	80.8	80.8	80.6	80.4	79.0	77.4	80.2
Los Angeles, Calif....	51.0	52.7	60.1	60.4	65.2	70.6	76.3	76.4	72.1	64.2	58.2	53.2	63.0
Mobile, Ala.....	49.8	53.2	59.1	66.0	75.6	79.1	80.5	79.7	76.5	61.1	57.5	51.5	66.1
Nagasaki, Japan.....	42.4	42.4	48.7	57.9	64.4	71.1	78.3	80.0	74.5	64.6	54.9	46.0	60.4

MONTHLY MEAN PRECIPITATION													
Manila, P. I.....	1.10	0.39	0.67	1.18	4.06	9.80	15.32	13.78	14.53	7.48	5.16	2.28	75.87
Los Angeles, Calif....	2.01	1.98	2.34	0.70	0.35	0.05	0.02	0.14	0.14	0.54	0.77	1.57	10.59
Mobile, Ala.....	4.86	5.06	7.17	4.35	4.00	5.95	7.04	6.81	5.02	3.18	3.74	4.57	62.04
Nagasaki, Japan.....	2.96	3.03	5.04	8.12	7.44	11.10	10.16	7.73	7.09	5.52	3.58	3.64	74.29

NUMBER OF RAINY DAYS													
Manila, P. I.....	5	3	3	4	9	16	21	21	20	16	12	8	138
Los Angeles, Calif....	6	6	7	4	3	1	0	0	1	3	2	6	40
Mobile, Ala.....	11	10	10	8	8	12	15	14	10	6	7	7	121
Nagasaki, Japan.....	16	13	14	15	14	16	14	13	13	11	12	16	165

While the season of 1916 was somewhat later than that of 1918 and more scab was noted, temperatures below those of 1918 were recorded during most of the development of the spring growth. By the time optimum temperatures for infection occurred growth was completed. However, the amount of growth was smaller and maturation of foliage was more rapid.

The lateness of the season in 1920 was due to the low temperatures which occurred during the first week of March. All the young growth formed before this time was killed back. The mean monthly temperature for March was also below normal. Thus the forcing of growth from new buds was resorted to by the plants, which naturally delayed the development of the first spring growth. However, temperatures very much like those which prevailed during 1915 occurred for the rest of the season, which were very conducive to maximum infection. Thus, during the season of 1920, scab was almost as bad as in 1915.

For practical purposes it appears that a mean monthly temperature below the normal for March in Alabama can be used as an indication of a scab epidemic, while temperatures above the normal for this month and with no freezing temperatures are indicative of a light scab season. The orchard grower then can forecast to some extent light or bad scab seasons from the mean monthly temperature and its departure from normal for March and can regulate his spraying program accordingly, depending on the prevailing conditions.

It might be stated at this point that there is not much use in spraying after the weekly mean temperatures go above 75° F. nor after the first growth period is completed.

## WEATHER AND ITS EFFECT ON THE DISTRIBUTION AND PREVALENCE OF CITRUS SCAB

In discussing the distribution of citrus scab it was stated that this disease was rather serious in the Gulf Coast States, Japan, and South China, while scab was not known in the Mediterranean countries, California, and the Philippine Islands, although scab had undoubtedly been introduced into California and possibly the Philippine Islands many times. It was further stated that there were certain factors which prevented the successful propagation of citrus scab in these localities.

To determine just what some of these factors might be, the mean monthly temperature and precipitation and number of rainy days for Manila, P. I.; Los Angeles, Calif.; Mobile, Ala.; and Nagasaki, Japan; were obtained.<sup>3</sup> These data are tabulated in Table II and plotted in figure 2. For the sake of simplicity, in the discussion which follows, all references to the relation of these environmental factors to the development of the host plants will be eliminated. It should be understood, however, that young growth must be present as one of the prerequisites for successful infection.

It can be readily seen that the principal reason why scab is not prevalent in the Philippines is the fact that the mean monthly temperatures for the whole year are above the maximum for infection. Even though scab was introduced on Citrus stock, the fungus could not propagate itself because of this fact. There is no doubt that citrus scab has been introduced into the Philippine Islands on nursery stock from Japan in the past, but owing to the prevailing high mean temperature militating against it, scab has never gained a foothold.

If we follow the temperature curve for Mobile through we find that some scab could occur in March, but that by the end of May temperatures are already above the maximum. The period of optimum infection would be rather short.

<sup>3</sup> Climatic data were kindly supplied by J. Warren Smith, agricultural meteorologist, Weather Bureau, U. S. Department of Agriculture.

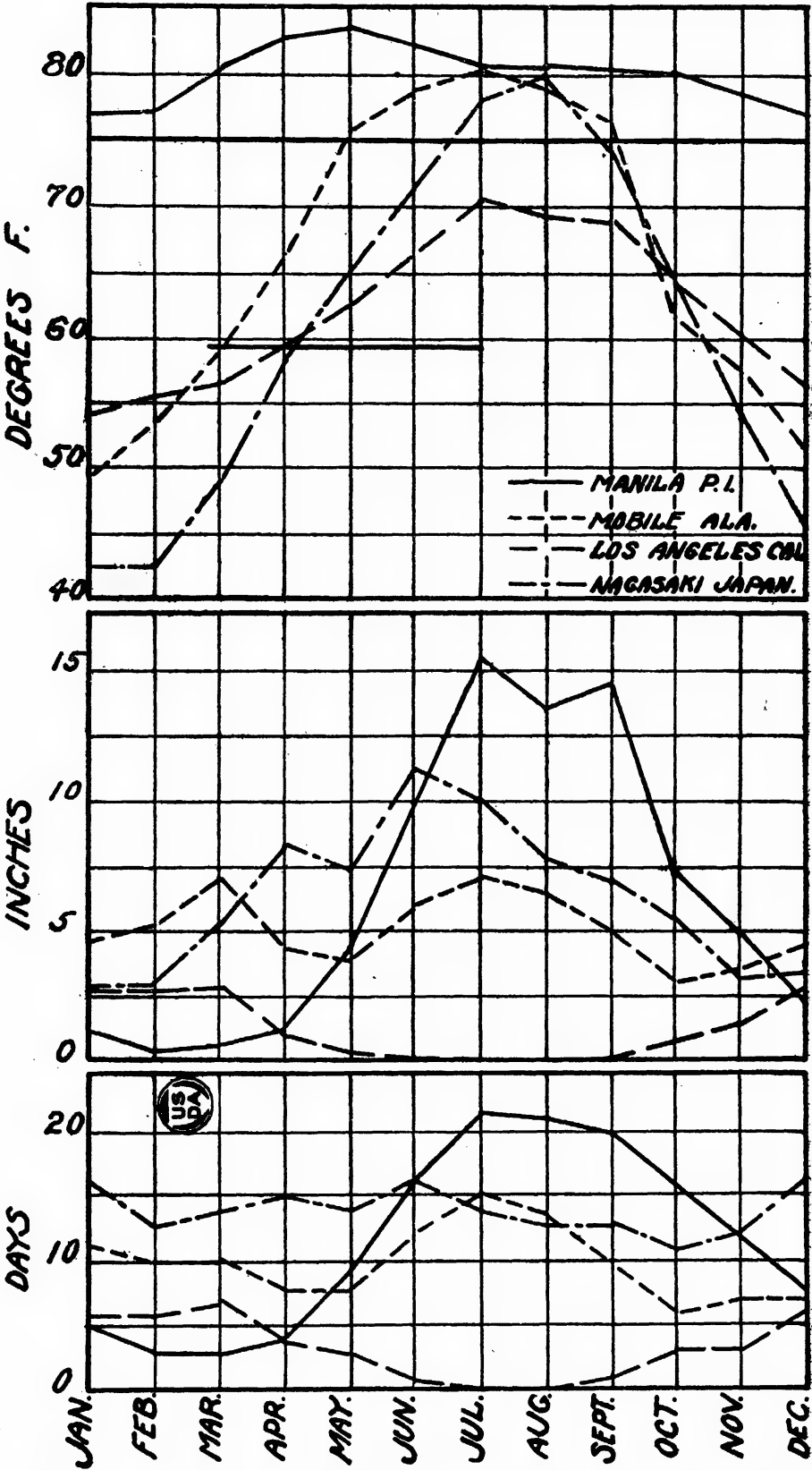


FIG. 2.—Graph showing the monthly mean temperatures, precipitation and number of rainy days for Manila, P. I.; Mobile, Ala.; Los Angeles, Cal.; and Nagasaki, Japan, together with the minimum and maximum temperatures for citrus scab infection



At the most, only the months of March, April, and May would be suitable for scab infection judging from the temperature factor alone. However, we should note that for these three months an average of approximately 5 inches of rain occurs during about ten days each month.

At Nagasaki, Japan, the monthly mean temperatures for the first five months of the year are from 8° to 11° lower than at Mobile. In other words, the season at Nagasaki is from one to one and one-half months later. Scab should be prevalent in Japan during the last of April, May, June, and part of July. Optimum conditions for scab infection should prevail during the month of June. During these months from 7 to 11 inches of rain occur during approximately 15 days each month. Scab is more serious in Japan than in Mobile district because of the slower start made by the plants in the spring, the larger amount of precipitation, and the greater number of rainy days.

After following through the temperature curve for Los Angeles, Calif., we would expect that as far as this one factor was concerned scab would be more serious than in either Japan or Alabama. Note that the curve follows that of Mobile for the first three months and then crosses over and runs along that for Nagasaki through July. We should expect then from the temperature standpoint alone to have conditions favorable for scab during the months from March through July. However, one need but look at the amount of precipitation to determine why scab can not propagate itself in California. This is a further indication that generous precipitation over most of the year is necessary for the development and propagation of scab.

The absence of scab in a locality may be due either to a mean temperature too high for the pathogene to infect the host or to a deficiency in rainfall. In the Philippine Islands the high mean temperature and possibly the effects of the dry season are a sufficient barrier to prevent the spread and development of scab, while in California the large deficiency in precipitation alone is sufficient.

The factors necessary for this disease to develop in any locality are a suitable temperature, sufficient moisture, and young growth. Wherever these conditions are fulfilled as in the Gulf Coast States and Japan, scab becomes a serious disease. No doubt, as has been pointed out before, we may have epidemic and nonepidemic years, depending on the prevailing weather, but some scab is present each season.

## DISCUSSION

In the foregoing pages the relation of environment to the various phases of the development of citrus scab, from the standpoint of experimental results obtained in the laboratory and greenhouse and from observations made in the field, are discussed. The temperature to which the pathogene and the plants were submitted under controlled conditions have been constant and not fluctuating as occurs in nature. Observations made under field conditions in south Alabama, where the Citrus-growing area is small and rather compact, when correlated with results obtained under controlled conditions are in close uniformity and the interpretations placed on these correlations can be made specific. Note also that we have confined ourselves to the relation of the scab during the development of the first spring growth. It is usually only during this period that scab does any serious damage. Occasionally scab appears in the fore part of June, late in August, or September on the young growth, when there are periods of several days during which the mean temperatures and other factors suitable for infections are favorable.

In the discussion of the effects of weather on the distribution and prevalence of scab in the other Citrus-growing regions we have tried to point out in a general way that there are certain types of weather which either favor or inhibit the

development of scab. Obviously one could not without a close study of the conditions prevailing in an entire Citrus-growing region state positively that the whole region would be free from scab, from the weather data obtained from one locality in that region. The exact correlation of environment to the development of scab in a Citrus-growing area must be left to trained observers present in that locality.

### SUMMARY

1. Citrus scab, caused by *Cladosporium citri* Masee, has been encountered with frequency during citrus-canker investigations made by the writers in Alabama during the past four years.

2. The disease is probably indigenous to the Orient, and has been distributed directly or indirectly from Japan to the Citrus-growing regions where it is not prevalent.

3. Citrus scab is a rather serious disease in the Gulf Coast States of Florida, Alabama, Mississippi, Louisiana, and Texas; in South China, Japan, and the West Indies. Scattering reports of citrus scab have also been made from South Africa, Australia, Formosa, Yucatan, Paraguay, Hawaii, India, and the Canal Zone. As yet, no scab has been reported from California, the Philippine Islands and the Citrus regions of the Mediterranean.

4. The three essentials for successful infection of Citrus plants by *Cladosporium citri*, under controlled conditions, are the presence of free moisture, young growth, and temperatures between 15° and 23.5°C. (59° and 74.3°F.). The optimum for the best development of scab appears to be about 20°–21°C. (68°–70°F.).

5. Under Alabama conditions, temperatures favorable to optimum infection usually prevail during parts of April and May. Sufficient moisture is generally at hand during this interval for successful infection to take place. The most important and variable factor is the development of the early spring growth.

6. Susceptible species or varieties of Citrus making a slight or slow spring growth which matures rapidly are not subject to scab. Likewise, those plants which do not start growth until late are also free from scab. In both instances it is apparently a question of escape. Plants making a large amount of spring growth, with slow maturation of foliage are easily infected and as a rule badly attacked.

7. Any environmental factor or factors including a slight spring growth and rapid maturation or late starting, favors scab escape; while any environmental factor or factors inducing a large amount of spring growth and slow maturation, favors scab susceptibility.

8. We must consider not only the internal factors influencing the development of spring growth of Citrus plants, but also the external factors which may inhibit or stimulate these processes.

9. Conditions essential for an epidemic scab year in Alabama depend to a large extent on a late season, coupled with sufficient moisture and the development of spring growth at the time optimum temperatures for infection prevail. An early season is favorable to scab escape, in that first growth is about completed when optimum conditions for infection are at hand.

10. Under Alabama conditions it appears that a light or bad scab year can be predicted to some extent by the mean temperature prevailing in March; a temperature below normal indicates a bad scab season; a temperature above normal, a light scab year.

11. The relation of weather and its effect on the distribution and prevalence of citrus scab is shown and the absence of scab in certain localities is correlated with either a mean temperature too high for infection or a deficiency in rainfall.

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# A STUDY OF ENSILING A MIXTURE OF SUDAN GRASS WITH A LEGUME<sup>1</sup>

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## OUTLINE OF EXPERIMENT

This paper reports a study of ensiling a silage crop high in protein and low in carbohydrates, mixed with one low in protein and high in carbohydrates, to determine whether such a mixture makes better silage than the same crops ensiled separately.

Two legumes, soybeans and cowpeas, were the high-protein crops used, and Sudan grass was the low-protein, high-carbohydrate crop.

Experimental silos were filled with each of the crops alone, with a mixture of Sudan grass and soybeans, and with a mixture of Sudan grass and cowpeas. The silage was rated on the basis of keeping quality, palatability, chemical composition, and loss in food constituents.

## PREVIOUS INVESTIGATIONS

It is a common practice in many sections of the country to mix together two different silage crops, one as supplement to the other, supplying in greater amount what the other lacks in certain feed constituents. The usual combination is a material rich in fermentable carbohydrates but low in protein, like corn; with one rich in protein, like the legumes. Typical combinations are oats and peas, corn and soybeans, sweet sorghum and alfalfa.

Sudan grass, soybeans, and cowpeas have all been ensiled, both alone and in combination with other crops. Sudan grass has been ensiled alone by Francis and Friedemann<sup>2</sup> at the Oklahoma Experiment Station; Sudan grass, soybeans, and cowpeas have been ensiled alone with success at the Missouri Experiment Station by Eckles,<sup>3</sup> and Sudan grass has been ensiled with rape with satisfactory results by Lamb and Evvard<sup>4</sup> at the Iowa Experiment Station. Soybeans have been ensiled with corn frequently, but most recently by Dvorachek and Mason<sup>5</sup> at the Arkansas Experiment Station, who state that a silage 10 per cent more valuable was obtained by drilling in soybeans with corn and ensiling them together than by using corn alone.

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<sup>1</sup> Received for publication April 18, 1924.

<sup>2</sup> FRANCIS, C. K., and FRIEDEMANN, W. G. SUDAN GRASS SILAGE. Okla. Agr. Exp. Sta. Bul. 115, 8 p., illus. 1917.

<sup>3</sup> ECKLES, C. H. LEGUMES, SUDAN GRASS AND CEREAL CROPS FOR SILAGE. Mo. Agr. Exp. Sta. Bul. 162, 25 p. 1919.

<sup>4</sup> LAMB, A. R., and EVVARD, J. M. RAPE AS MATERIAL FOR SILAGE. Jour. Agr. Research. 6: 527-533. 1916.

<sup>5</sup> ARKANSAS AGRICULTURAL EXPERIMENT STATION. BETTER SILAGE BY ADDING A LEGUME. Ark. Agr. Exp. Sta. Bul. 181: 60-61. 1922.

## EXPERIMENTAL WORK

## FIELD METHODS

The Sudan grass, soybeans, and cowpeas were sown on June 3, in separate plots of less than an acre, on land previously used for corn at the United States experiment farm at Beltsville, Md. The first two made good growth, but the stand of cowpeas was very thin and spindling. They were all harvested September 2, when the Sudan grass was in the dough stage and the soybeans and cowpeas were well podded. All the Sudan grass and the part of the soybeans which was ensiled with it were hauled to the cutter fresh; but the remainder of the soybeans, which was ensiled alone, and all the cowpeas were allowed to wilt before being hauled from the field.

The experimental silos used were wooden tubs 2 feet in diameter and 4 feet deep. They were provided with wooden followers fitting as closely as possible within the silo walls. Pressure of 100 pounds per square foot, to approximate the pressure within a large silo, was obtained by means of a lever and weight applied to the center of the follower. The silo was filled to within 16 inches of the top, and a piece of cheesecloth was then carefully spread over to make a complete separation between the portions of silage below and above. The portion below the cheesecloth was called the experimental portion. The remainder of the silo was then packed with the same kind of silage material, another piece of cheesecloth was spread on, and the follower, lever, and weight were adjusted in place.

Five of these silos were used. They were filled as follows: (1) Sudan grass alone; (2) Sudan grass and soybeans, half-and-half; (3) wilted soybeans alone; (4) wilted cowpeas alone; (5) Sudan grass and wilted cowpeas, half-and-half mixture. Samples were taken at the time of filling for laboratory analyses.

Three months after ensiling, on December 3, all five silos were opened. The top portions down to the cheesecloth separators were weighed and discarded. The remainder, or experimental part, in each silo was weighed, thoroughly mixed, and a portion of the well-mixed lot taken as a sample for laboratory analysis. After the laboratory samples were taken, the remaining portions of the silages were used as material to conduct comparative palatability tests.<sup>6</sup>

## METHODS OF ANALYSIS

A 1-kilogram subsample from a thoroughly mixed field sample of each kind of silage was dried in a steam-heated drying closet at about 55° C. to air-dry weight. These subsamples were then ground in an electric pulverizing mill so as to pass a 40-mesh sieve. Moisture, total protein, albuminoid protein, ash, crude fiber, and ether extract were determined on the finely ground sample according to methods outlined in "Methods of Analysis" by the Association of Official Agricultural Chemists.<sup>7</sup> Moisture was found by adding the weight of moisture lost in obtaining the air-dry sample to that lost on drying the air-dry sample, and calculating as a percentage of the original moist material.

<sup>6</sup> Acknowledgment is made to H. T. Converse for suggesting and conducting the palatability test and summarizing the results.

<sup>7</sup> ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. As compiled by the Committee on Revision of Methods. Revised to Nov. 1, 1919. p. 71-99. 1920.

TABLE I.—*Loss or gain in weight of experimental portion of silage*

Kind of silage	Experimental portion		Loss (—) or gain (+)
	Put in	Taken out	
	<i>Pounds</i>	<i>Pounds</i>	<i>Per cent</i>
Sudan grass alone.....	198. 0	198. 5	+0. 25
Sudan grass and soybeans.....	198. 0	196. 0	—1. 0
Soybeans alone.....	207. 0	209. 5	+1. 2
Cowpeas alone.....	246. 0	247. 0	+0. 4
Sudan grass and cowpeas.....	185. 0	184. 5	—0. 3

## DISCUSSION OF RESULTS

There was very little apparent change in weight on the experimental portion of any of the silos. Except in one case, that of the Sudan grass alone, all the experimental portion was excellent silage, and in most cases some of the filler portion also. The Sudan grass alone did not pack well, so that not only was all the filler spoiled, but also about 10 pounds of the experimental portion were moldy. So far as one could judge by taste and smell of the silage, that in one silo kept as well as another, or nearly so. In general appearance, however, the mixtures made much better silage than the Sudan grass. On the other hand, the mixtures, while yielding excellent silage, did not seem to yield any better silage than the legumes alone. As suggested by Eckles,<sup>8</sup> the Sudan grass and the cowpeas were allowed to wilt before being cut for the silo, and, as he found, a perfectly normal and very palatable silage was produced.

## PALATABILITY

The palatability test conducted with the different silages furnished a very interesting and practical basis of comparison.

If the palatability of the silages is to be judged by the way in which the cows ate them at the start, the Sudan grass had little influence, if any, in either increasing or decreasing the palatability of the legumes, even though the Sudan-grass silage alone was quite unpalatable. The final conclusion reached as a result of the palatability test was that the soybean silage was very slightly more palatable than the cowpea silage; that the legumes were very slightly more palatable than the mixtures; that there was no appreciable difference between the mixtures; and that Sudan-grass silage alone was far less palatable than any of the other silages.

## CHEMICAL COMPOSITION

Table II gives the composition of the silage crops as they were put into the silo and of the silages as they were taken out.

These data show that dry matter does not vary much between silos except in the case of the soybeans alone, which is probably due to wilting. As might be expected, the percentages of total and albuminoid proteins are considerably greater in the mixtures than in the Sudan grass and less than in the legumes. Ash seems to be highest in the cowpea silage and lowest in the Sudan grass alone. Crude fiber does not vary much between samples except in the case of cowpeas alone, where it is considerably lower. Ether extract does not vary much except with the soybeans alone, where it is somewhat lower than in the other silage. Nitrogen-free extract is highest in the Sudan grass and lowest in the soybeans alone, though it must be said the differences are not remarkable.

<sup>8</sup> ECKLES, C. H. LEGUMES, SUDAN GRASS AND CEREAL CROPS FOR SILAGE. Mo. Agr. Exp. Sta. Bul. 162, 25 p. 1919.

TABLE II.—Composition of silages as put into and taken out of silos

Kind of silage	Dry matter in moist material		Water-free basis											
			Total protein		Albumi- noid protein		Ash		Crude fiber		Ether extract		Nitrogen- free extract	
	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Sudan grass alone.....	30.3	28.9	6.0	6.4	5.3	4.5	5.5	6.1	34.0	35.2	1.5	1.9	53.0	50.4
Sudan grass and soy- beans.....	30.3	30.1	11.0	11.3	9.0	7.1	6.8	7.5	31.6	34.0	1.4	2.3	49.3	44.9
Soybeans alone.....	36.3	34.8	16.7	17.6	12.7	9.8	6.7	8.3	30.5	32.7	1.2	2.0	44.9	39.5
Cowpeas alone.....	31.8	31.0	16.0	15.9	13.4	9.9	7.7	8.8	23.8	27.1	1.5	2.6	51.0	45.6
Sudan grass and cowpeas.....	33.3	30.6	11.8	12.3	9.8	8.9	7.1	8.1	29.9	30.6	1.7	2.5	49.6	46.6

Table III gives the percentage of loss or gain in different feed constituents of the silage, based on the weights of each put in and taken out of the silo. Gain in weight of any of the feed constituents can be explained only by down wash from the filler portion of each silo of portions of these constituents. The loss or gain of weight in moisture and dry matter as shown in Table III is not remarkable in any of the silages. Sudan grass and cowpea mixture shows the greatest loss of dry matter, followed by Sudan grass alone. As usual, in silage there is a large loss of albuminoid protein due apparently to the breaking down of the more complex protein into simpler nitrogen compounds. These simpler nitrogen compounds are generally considered to have less feed value than the original albuminoids. The losses of albuminoids are larger in each legume silage alone than when Sudan grass was ensiled with this legume. This would indicate that a considerable loss of feeding value was prevented by the presence of the Sudan grass.

The ash shows an unusual gain in all cases, from 5 per cent in the Sudan grass silage to 21 per cent in the soybean silage, and 23 per cent in the Sudan grass and cowpea mixture. These gains correlate very well with the gains in amount of moisture, and may be due to a down wash of soluble ash from the filler portion of the silage which lay above the experimental portion. Crude fiber shows a gain of 9 per cent in the cowpea silage and a gain of nearly 6 per cent in the Sudan grass and soybean mixture, while in Sudan grass silage and the Sudan grass and cowpea mixture, losses are indicated. Ether extract shows gains of from 7 per cent in the cowpea silage to 54 per cent in the Sudan grass and soybean mixture. There is a loss of nitrogen-free extract in every silage, varying from 9 per cent in Sudan grass silage to 15 per cent in soybean silage. The loss of nitrogen-free extract and therefore of feeding value is somewhat larger in the soybean silage than in the soybean and Sudan grass mixture. No such difference is shown between the cowpea silage and the cowpea and Sudan grass mixture.

TABLE III.—Loss (–) and gain (+) in weight of silage constituents as put into and taken out of silos

Kind of silage	Green mate- rial	Mois- ture	Dry matter	Total protein	Albu- minoid protein	Ash	Crude fiber	Ether extract	Nitro- gen-free extract
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Sudan grass alone.....	+0.25	+2.2	–4.3	–1.8	–19.3	+5.2	–1.2	+23.9	–9.0
Sudan grass and soybeans ...	–1.0	–0.7	–1.8	+1.4	–22.7	+8.5	+5.8	+54.0	–10.5
Soybeans alone .....	+1.2	+3.7	–3.2	+1.7	–25.4	+21.0	+3.9	+52.2	–15.0
Cowpeas alone.....	+0.4	+1.5	–1.9	–2.3	–27.7	+11.1	+9.1	+7.3	–12.3
Sudan grass and cowpeas.....	–0.3	+3.6	–8.1	–4.0	–17.0	+23.0	–6.0	+33.5	–13.9

## CONCLUSIONS

There was little difference in keeping quality among the silages studied. The legumes ensiled alone produced as normal a silage as the mixtures. Sudan grass produced a silage which appeared as normal as the others, but as it did not pack so firmly it yielded a larger amount of spoiled silage.

The legumes were slightly more palatable than the mixtures, and the Sudan grass was very much less palatable than the rest of the silages.

Outside of the Sudan grass silage, which was very low in protein, and soybean silage, which was low in nitrogen-free extract, the differences in chemical composition did not indicate any marked superiority of one silage over another. However, if there was any silage better than another on the basis of chemical composition, it was the cowpea silage. The mixtures were practically as good as the legumes, except in protein content, but no better.

The legumes showed a greater actual loss of albuminoid protein and therefore of feeding value than the mixtures. Soybean silage showed a greater loss of feeding value in nitrogen-free extract than the soybean-Sudan grass mixture. Sudan grass alone showed the least loss of these valuable feed constituents.

It does not seem necessary, from this study, for the production of good silage to ensile together a crop high in protein with one high in carbohydrates. The crop carrying a large percentage of carbohydrates usually takes care of itself in the silo; and the high-protein crop, if wilted so as to contain the proper moisture content, also produces a satisfactory silage.





# BACTERIAL LEAFSPOT OF DELPHINIUM<sup>1</sup>

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## INTRODUCTION

The leafspot of Delphinium (larkspur), commonly known as the "Black spot," or "Black disease," is a very destructive disease, widespread throughout the northern United States, more particularly in the North Atlantic States. Although this decorative plant has been eliminated from the gardens of some large estates because of its ravages, the disease has received scant attention from pathologists.

The only references in pathological literature are brief notes by Dr. Erwin F. Smith. In "Science," for March, 1904,<sup>2</sup> he gave a very brief description of the causal organism which he had isolated, proved pathogenic by inoculations and named *Bacillus delphinii*. Cultures were allowed to die before adequate cultural work was done, and although the organism was again isolated in 1907, pressure of other work crowded out its further study. The spots are figured by him in "Bacteria in Relation to Plant Diseases,"<sup>3</sup> and in the same volume, page 92, it is stated to be one of the diseases transmitted through stomata and water-pores. He later<sup>4</sup> refers again briefly to the subject, as follows:

"The spot disease of Delphinium (Vol. I, fig. 127) is another malady in which infection takes place readily through the unbroken leaf-surface and stem-surface, i. e., through stomata. The disease has been obtained a number of times during the last seven years by placing the bacteria in water and spraying this upon the plants. The leaf-serratures also blacken in this disease, and here infection probably occurs through the groups of water pores situated on their apex."

In 1920 characteristically spotted leaves were received from Woodstock, N. Y., from which isolations were made of a white bacterial organism. These and subsequent isolations from various sources have been used for the study here presented.

## HOST PLANTS

This disease has not been reported on any plant except larkspur, being most destructive on the choice hybrid delphiniums. A few small but definite infections have been produced on Aconite by rubbing the inoculum on the lower surface of leaves, but no natural infections have been reported on this plant. No infections were obtained by repeated inoculations on cultivated varieties of *Ranunculus* and *Aquilegia*.

## GEOGRAPHICAL DISTRIBUTION

Specimens have been received from points in Maine, New Hampshire, Massachusetts, Connecticut, New York, Long Island, Pennsylvania, Illinois, and in one case from Portland, Oreg. One correspondent wrote in 1922, "I have just

<sup>1</sup> Received for publication Jan. 23, 1924.

<sup>2</sup> SMITH, E. F. BACTERIAL LEAF SPOT DISEASES. *Science* 19: 417. 1904.

<sup>3</sup> SMITH, E. F. BACTERIA IN RELATION TO PLANT DISEASES. v. 1, fig. 127. 1905.

<sup>4</sup> SMITH, E. F. BACTERIA IN RELATION TO PLANT DISEASES. 2: 61-62. 1911.

returned from a trip and find that the delphinium all the way from Ridgefield, Conn. out to Chicago, Ill., is badly affected by the 'black disease.' " A delphinium grower and breeder in California reported in 1923 that the "Black disease" which gives so much trouble in the East had not as yet appeared or seemed not to be known on the West Coast.

Leaves from Castine, Me., received early in July, 1922, were 23 cm. across and came from plants 3 meters high. They were thickly covered with the largest spots observed, i. e., 1 to 2 cm. in diameter (Pl. 1, B). Probably the cool, moist climate in this location which was a large factor in the vigor of these plants also increased the virulence of the disease. On less vigorous plants the spots are usually smaller (Pl. 1, A). No specimens have been received from farther south than Pennsylvania, and inoculations in Washington, D. C., where the climate does not favor this plant, while giving numerous typical infections, have never produced spots more than a few mm. wide (Pl. 1, C). During the heated summer term it has been found impossible to produce artificial infections in Washington, D. C., except in the event of a cool, rainy spell.

### APPEARANCE OF DISEASED PLANTS

The leafspots are very striking in appearance. On the upper surface they form tarry black areas of irregular shape and size that may reach a diameter of 2 cm. on large vigorous leaves (Pl. 1, B). On the lower surface they are brown and the smaller ones do not yet show through to the upper side. Old spots lack the water-soaked edges that so frequently accompany other bacterial spots, although in very young stages water-soaking is present and is the first indication of infection observable under a hand lens. Although the lower surface of spots may appear sunken, the upper surface usually appears to be raised slightly and, in many spots, there is a tendency to concentric rings strongly suggesting fungus infection (Pl. 2, D and E). Such spots were repeatedly sectioned to make sure that no mistake was being made, but in every case bacteria oozed out in vast quantities, while no fungus was observed and cultures of *Bacterium delphinii* were obtained when poured plates were made. Spots resulting from pure culture inoculations also often showed this zoning, which has since been noted in bacterial spots on other plants, notably tobacco.

Spots may occur on any part of the leaf blade, the result of stomatal infection, and are also common on the leaf tips where they make their entrance through the large water-pores (Pl. 2, A, B, and C). When infection takes place on young leaves, and especially in the deep sinuses, distortion usually results from the failure of the diseased areas to keep pace with the growing healthy tissue. Flower buds are also occasionally attacked, becoming black-spotted and distorted, but this should not be confused with the common distortion of buds and young leaves caused by mite infestation where a blackening of the very young tissues often occurs in severe cases. This is a continuous blackening instead of a spotting and no bacteria are present. The bacterial spot occurs also on petioles and stems. In later stages of the disease the spots may coalesce, forming large black areas, involving in some cases almost the entire blade of the leaf.

### HISTOLOGY OF DISEASED LEAVES

The bacteria enter the leaf through the stomata of the lower epidermis or through the water pores at the tips of the serratures. As the spongy parenchyma just beneath this epidermis is very loose, it is difficult to demonstrate stomatal infection in its earliest stages, since the bacteria scatter so readily on entering. Also, unless sections are made early enough the infection spreads so rapidly as

to obscure any evidence of the point of entry. Material fixed 24 hours after spraying gave no sections suitable for photographic demonstration although the spots were water-soaked and scattered bacteria were found under the stomata in microtome sections. After 48 hours, however, very good sections were obtained showing bacteria in the intercellular spaces under the stomata chiefly lying on the cell walls, singly or in oval masses (Pl. 3, A and B). At this stage the spot shows as a minute water-soaked point on the lower surface, to be seen only under a lens. In microtome sections the bacteria are confined to the loose parenchyma. Cells in the vicinity of the invaders often show on their walls what appear to be extrusions of cell sap. These are hemispherical bodies which stain very deeply (Pl. 4, A). Later the palisade tissue is invaded and killed and the lesion penetrates to the upper surface as a black spot involving the whole thickness of the leaf. At this stage of infection the bacteria lie on the walls of the cells and crowd the intercellular spaces.

### ISOLATIONS

Poured plates were made in this laboratory in 1903 from spotted leaves from New York and Massachusetts and again in 1907 from Massachusetts. In each case a white organism was obtained with which successful infections were secured on delphiniums. On account of the small amount of cultural work done at that time no adequate comparison can be made with isolations of 1920-1923. Descriptions and photographs of agar plate colonies agree with those of later date but in other cultural characters there is disagreement, not only between these early isolations and those of 1920-1923 but also among the several early isolations themselves, and it is apparent that some of the early cultural work was not fully checked up, since striking inoculations were obtained in 1903 proceeding from subcultures of single poured-plate colonies of what was undoubtedly this organism.

Isolations have now been made from typical infected plants from all of the States mentioned under "Geographical distribution." All gave colonies apparently of the same white organism. Comparative cultures have shown these to be alike in all important points.

In several cases where material was not in good fresh condition when received a yellow organism appeared on the plates in numbers about equaling the white colonies and, in one case, only a yellow organism was obtained although three separate platings were made from typical spots. This was of a saprophytic type frequently appearing on plates poured from many species of plants when not in perfectly fresh condition, and it failed to produce any infections when the inoculum was either sprayed or rubbed on the lower surface of delphinium leaves.

### INOCULATIONS

Successful inoculations have been made in the hothouse and out of doors, on young and on mature plants, both in cages where moisture could be maintained and also without cover. Infections obtained on young plants in the hothouse never increased much in size (1 or 2 mm. wide) and few secondary infections were found. Reisolations were made from hothouse inoculations of 1920 and used for inoculations in the hothouse in 1921 and 1922 with similar results. Outdoor inoculations in the summer of 1921 failed, that is only a very few slow-growing infections were secured, probably because of the late start made and supervening dry, hot weather.

On May 31, 1922, vigorous young seedling plants of hybrid delphiniums, which were set out of doors May 1 and were just beginning to send up blossom stalks, were sprayed with subcultures from an isolation of March 29, 1922, from a hot-

house infection. It was a cool cloudy day with threat of rain and the plants were left uncovered. They were sprayed with the organism on two successive cloudy days, and then a three days' continuous rain set in. Nine days after the first spraying the leaves were thickly speckled with infections, especially those inoculated from one colony, individual spots being as much as 2 mm. wide and some confluent areas as large as 1 cm. across. These spots were black, surrounded by yellow areas on some leaves while on others (older mature stem leaves) no yellowing occurred. Poured plates from such spots gave pure cultures of the white organism used for inoculation. The weather then turned very warm, the spots did not increase materially in size and later attenuated. Inoculation failed, both with reisolations from these leafspots and a copious colonies used to produce them. It seems evident that climatic conditions. After 3 cold and moisture, play a large part in the success of inoculation. High temperatures inhibit, and moist cool conditions greatly favor the disease.

During April and May, 1923, several sets of inoculations on delphinium plants that had wintered within 4 or 5 days and increases in 1921 which had produced the infections are moderately clouded. After 3 months' incubations of 1922 were used. Absence of white precipitate in the bottom of the tube with numerous small crystals.

**USCHINSKY'S SOLUTION.**—Within 24 hours a faint clouding may be seen in the upper part of the culture. By the second day a delicate pellicle has formed with blue-fluorescence just below it in the upper 2 or 3 mm. and by the fifth day clouding is heavy with a heavy pellicle and green fluorescence throughout. A heavy white precipitate is formed.

**FERMI'S SOLUTION.**—There is prompt growth in Fermi's solution with a beautiful blue fluorescence sometimes becoming green but more often remaining blue though held for several weeks. A heavy wrinkled pellicle is formed which does not fall readily on shaking, but clouding usually remains weak. Thumm<sup>7</sup> states that all of the colors produced by fluorescent bacteria are due to the same pigment, the blue becoming green with the production of alkali by the organism.

The addition of dilute ammonia to blue cultures of *Bact. delphinii* in Fermi's solution turned them a vivid green. Blue and green cultures were tested for relative acidity with the following results:

Blue  $P_H$  6.5 or +41.

Green  $P_H$  6.6 or +34.

**NITRATE REDUCTION.**—Nitrate is not reduced. Tests were made with nitrate bouillon cultures 5 days old and 10 days old in which moderate clouding had taken place, using the starch-iodin-sulphuric acid test.

**NITROGEN COMPOUNDS.**—The ability of the organism to obtain its nitrogen from various nitrogen compounds was tested in 1 per cent water solutions of the following: peptone, asparagin, asparagin plus dextrose, ammonium citrate, ammonium tartrate and ammonium succinate. The results are shown in Table I.

<sup>6</sup> RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. pl. 15. Washington, D. C. 1912.

<sup>7</sup> THUMM, K. BEITRÄGE ZUR BIOLOGIE DER FLUORESCIERENDEN BAKTERIEN. Arb. Bakt. Inst. Tech. Hochschule Karlsruhe 1: 291-377. 1897. Abstract in Smith, E. F. Bacteria in relation to plant diseases. 1: 238. 1905.

## CULTURAL CHARACTERS

**BEEF AGAR PLATES.**<sup>5</sup>—Colonies are visible on the second day and by the third day in thin sown plates are 3 to 5 mm. wide, round, white, transparent, opalescent, slightly convex, sometimes slightly umbonate, smooth shining, finely granular. Young colonies show coarse cross-hatching by oblique transmitted light (Pl. 4, F and G) and sometimes these internal cross-hatchings persist in the older colonies at summer temperatures but usually they give place to internal concentric markings (Pl. 4, E). Buried colonies are lenticular. When older, the colonies may reach a diameter of 8 mm. The consistency is sometimes slightly viscid. Occasionally a ring of irregular opaque areas or radiating lines may appear in the colonies (Pl. 4, D) and old colonies may become slightly lobed. Plates from old litmus milk and from old bouillon cultures have given very convex colonies with depressed centers which, as they enlarge, have a wrinkled surface. After return to favorable cultural conditions, transfers from these colonies again gave normal colonies on agar plates.

**BEEF AGAR SLANTS.**—Growth is thin, white, smooth shining, transparent, opalescent, with internal wavy markings and entire margins. Growth does not cover the surface but tapers upward from a width of 3 to 6 mm. There is considerable white precipitate in the V. Numerous small crystals form beneath the growth and the agar becomes green.

**POTATO CYLINDERS.**—The moderate, thin, spreading growth on potato cylinders is dirty white becoming pale tan colored, with a slimy consistency; the potato begins to gray in 24 hours and in a few days is gray throughout. There is no distinct odor. Starch is only partially digested, i. e., a purple color is produced by adding iodine to the crushed potato cylinders on which the organism has been growing.

**STARCH AGAR PLATES.**—Smears were made on starch agar poured plates from young agar cultures. When tested after 7 days by drenching the surface of the plate with iodine solution an area 5 to 8 mm. wide surrounding the growth was purple in color but there was no area of complete starch destruction.

**WHEY AGAR.**—On whey agar titrating +8 or  $P_H$  7.4 colonies are very convex, creamy white, opaque, round with entire margins, not opalescent, with internal concentric markings in the margins and in thin sown plates are 5 to 7 mm. wide when 10 days old.

**BEEF BOUILLON.**—Clouding occurs near the surface in 24 hours with a delicate pellicle which falls readily in fragments on being disturbed. Undisturbed cultures 2 days old are well clouded throughout and have a heavy pellicle, borne down in the center with a mass of white growth, giving the appearance of gelatin liquefaction (Pl. 4, H) when viewed from the side, but from the top showing a nail-head of white in the center of the membranous pellicle. This pellicle falls readily as a whole on shaking. Disturbed cultures re-form their pellicle quickly when young but not after the cultures are 6 or 8 days old. Blue-green fluorescence begins at the top in young cultures. Old cultures are green throughout.

**BEEF GELATIN PLATES.**—In +10 ( $P_H$  7.2) beef gelatin, kept at 20 to 22° C., colonies on the second day are minute white spots lying in shallow pits of liquefaction several times their width. By the fourth to the sixth day colonies may reach a diameter of 1 cm., clouding the liquefied gelatin uniformly except for a heavier mass of growth at the center.

**BEEF GELATIN STABS.**—Liquefaction begins on the second day (20 to 24° C.) and is napiform, becoming stratiform. Liquefaction is complete in two to three

<sup>5</sup> All beef media used were made with beef infusion plus 1 per cent Difco peptone. Titrations expressed by + and - are according to Fuller's scale.

weeks with deep green fluorescence. Cultures kept one year on culture media liquefy gelatin very slowly.

**MILK.**—Milk clears uniformly without coagulation, beginning about the seventh day. Cultures one month old are translucent and honey yellow. No crystals are formed at this time but an occasional culture held for three months has a few small masses of tyrosin crystals. The milk at this age is tawny yellow and translucent (Ridgway XV)<sup>6</sup> and the precipitate has become yellow.

**LITMUS MILK.**—Litmus milk begins to blue on the third day from the top downward, then clears without coagulation. The cream rim when present is reddened. Reduction of the litmus begins in 10 days and is complete in 20 days. A copious white precipitate is formed. The blue color returns in 2 to 4 weeks. After 3 months the milk is still blue and translucent but the precipitate has become yellow.

**METHYLENE BLUE MILK.**—Reduction begins on the second day and is complete in 3 to 5 days. No coagulation occurs.

**COHN'S SOLUTION.**—Weak clouding is visible within 4 or 5 days and increases slowly for 5 or 6 weeks when the cultures are moderately clouded. After 3 months' growth there is a small amount of white precipitate in the bottom of the tube with numerous small crystals.

**USCHINSKY'S SOLUTION.**—Within 24 hours a faint clouding may be seen in the upper part of the culture. By the second day a delicate pellicle has formed with blue-fluorescence just below it in the upper 2 or 3 mm. and by the fifth day clouding is heavy with a heavy pellicle and green fluorescence throughout. A heavy white precipitate is formed.

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TABLE I.—*Sources of nitrogen*

	1 day old	4 days old	10 days old	20 days old
Peptone.....	Very weak clouding.	Good clouding, heavy pellicle.	Same as 4 days, some precipitate.	No further growth.
Asparagin + dextrose.	.....do.....	Weak clouding, delicate pellicle.	Moderate clouding, good pellicle.	Do.
Asparagin.....	.....do.....	.....do.....	Weak clouding, delicate pellicle.	Do.
Ammonium citrate.	.....do.....	Weak clouding, no pellicle.	Weak clouding, no pellicle.	Do.
Ammonium succinate.	.....do.....	.....do.....	.....do.....	Do.
Ammonium tartrate.	No clouding.....	No growth.....	Very weak clouding....	Do.

**LITMUS SUGAR AGARS.**—Litmus agar containing 1 per cent peptone and 1 per cent of saccharose, maltose, lactose, dextrose, galactose, levulose, l. arabinose, glycerin or mannit was used for stroke cultures. The litmus is reddened decidedly on the second day and is red throughout in 6 days in the presence of dextrose, levulose, l. arabinose or galactose. Saccharose causes no color change except sometimes a very feeble reddening after 2 to 3 weeks. Cultures with maltose, lactose, glycerin and mannit become decidedly blue, beginning on the second day.

When the same carbohydrates are added to beef infusion peptone litmus agar the organism produces a blue reaction with all of them.

**FERMENTATION TUBES.**—In fermentation tubes containing 1 per cent peptone water and 1 per cent of saccharose, lactose, maltose, dextrose, galactose, glycerin or mannit, there is no growth in the closed end and no gas. In dextrose and galactose the reaction is acid to litmus while the others are alkaline to litmus. They were tested after 1 week, 2 weeks, and 4 weeks. Saccharose gives a neutral or slightly acid reaction.

**BLOOD SERUM.**—Only a very moderate growth takes place on Loeffler's blood serum and there is no liquefaction.

**TOLERATION OF SODIUM CHLORID.**—The organism grows promptly and well in 1 per cent NaCl in +10 or +12 beef bouillon and fairly well in 2 per cent, weakly in 3 per cent, and some isolations make very weak growth in presence of 4 per cent NaCl.

**OPTIMUM  $P_H$  IN BEEF BULLION.**—The best growth was made in +16 ( $P_H$  6.7) to +12 ( $P_H$  7.1). There was gradual but finally very good clouding in +25 ( $P_H$  5.6), but none in +30 ( $P_H$  5.2). Moderate clouding occurs in 0 ( $P_H$  8.2), weak clouding in -2 ( $P_H$  8.4) and in -4 ( $P_H$  8.6), but none in -9 ( $P_H$  9.2).

**TOLERATION OF ORGANIC ACIDS.**—Tests made with citric, malic, and tartaric acids are summarized in Table II. It was found that the  $P_H$  value is the controlling element here, the organism being able to grow in the same  $P_H$  of all acids while varying in its tolerance of the different acids judged by their Fuller's scale values.

**AMMONIA PRODUCTION.**—There is strong ammonia production. Cultures in beef bouillon 2 to 17 days old were tested by suspending over them strips of filter paper wet with Nessler's solution. Browning of the paper began immediately and became intense on heating the cultures in a water bath.

**HYDROGEN SULPHID PRODUCTION.**—No hydrogen sulphid is given off from bouillon, Uschinsky's solution, Fermi's solution, or potato cultures. Tests were made by suspending filter paper wet with lead acetate water in the tubes. No discoloration of the paper occurred.



TABLE II.—Toleration of organic acids

Citric acid			Malic acid			Tartaric acid		
Fuller's scale	P <sub>H</sub>	Growth	Fuller's scale	P <sub>H</sub>	Growth	Fuller's scale	P <sub>H</sub>	Growth
+17	.....	Prompt heavy clouding.	+17	.....	Prompt heavy clouding.	+17	.....	Prompt heavy clouding.
+28	5.7	Delayed heavy clouding.	+25	5.8	Delayed heavy clouding.	+24	5.7	Delayed heavy clouding.
+33	5.4	No growth.....	+33	5.2	No growth.....	+29	5.2	No growth.

INDOL PRODUCTION.—No indol is produced in 10 days' growth either in Dunham's solution or in Uschinsky's solution to which 2 per cent Difco peptone was added. Tests were made with sodium nitrite and sulphuric acid. Cultures of *Bacillus coli* tested at the same time gave a good pink reaction.

EFFECT OF DESSICATION.—The organism is rather resistant to drying. Small drops from 24-hour beef bouillon cultures were spread on sterile cover slips in sterile Petri dishes and kept in the dark. Most of the covers when dropped into tubes of bouillon after 9 days' drying caused clouding, many clouded the bouillon after 12 days' drying, and some were able to cloud the bouillon after 20 days' drying. Plates were poured from these last which gave pure cultures of the right organism.

THERMAL RELATIONS.—The organism grows at temperatures from 1° to 30° C. in beef bouillon +12, P<sub>H</sub> 7.1, with an optimum growth at 25°. At 1° clouding began on the sixth day and gradually (within three weeks) became heavy and green fluorescent with a pellicle. At 30° growth was very weak, even after three weeks, and without either pellicle or green color. Fresh transfers to bouillon kept at 34°, 35°, and 37° for eight days, when removed to room temperatures (23° to 25°) failed to cloud although held for two weeks.

The thermal death point is 50° C. when test-tube cultures in beef bouillon (P<sub>H</sub> 6.8 to 7.2) are exposed in a water bath for 10 minutes (tube diameter 16 mm.)

EFFECT OF FREEZING.—Transfers were made to beef bouillon (+12, P<sub>H</sub> 6.7) from 24-hour-old bouillon cultures. These were allowed to stand 10 minutes, shaking at intervals; then beef agar plates were poured. The freshly transferred cultures were then frozen solid in a salt and ice mixture, kept frozen for 15 minutes and thawed in cool water. Plates were then again poured as before. From 40 per cent to 60 per cent of the bacteria were killed by this treatment.

EFFECT OF SUNLIGHT.—Tests were made in Washington, D. C., in August. There were no clouds but the sky was not brilliantly clear. Plates poured from 24-hour bouillon were exposed bottom side up on ice to the direct rays of the sun. One-half of each plate was protected from the sun by several folds of black paper. Exposures were made for 5, 10, 20, 30, 45, and 60 minutes. A colony count gave the following results: One-third were killed by a 5-minute exposure, one-half by 10-minute, three-fourths by 20-minute and all by 45-minute and 60-minute exposures.

LONGEVITY.—The organism lives at room temperatures for six months in beef bouillon and in milk, or until the culture is actually almost completely evaporated.

GROUP NUMBER.—The group number is 211.2322123 according to the descriptive chart of the Society of American Bacteriologists.

## TECHNICAL DESCRIPTION

***Bacterium delphinii* (EFS).**

A short nonsporiferous rod with rounded ends; usually single or in pairs, sometimes in short chains; flagella 1 to 6 bipolar; capsules, aërobic, white, but producing a blue-green fluorescence in cultures; diastasic action weak; liquefies gelatin; does not reduce nitrates; clears milk without coagulation; blues, then reduces litmus milk; produces ammonia but no hydrogen sulphid or indol; forms acid from dextrose, galactose, levulose, and with more difficulty from saccharose, but not from lactose, maltose, glycerin or mannit; does not produce gas from any of these carbon compounds; grows well in Uschinsky's, Fermi's and Cohn's solutions; optimum temperature 25° C., maximum 30°, minimum 1°, or less; thermal death point 50°; Gram-negative; not acid-fast; stains readily with carbol fuchsin and gentian violet; is pathogenic to delphiniums, producing dead black spots on the leaves, stems and flower buds.

## CONTROL METHODS

It has been found impractical to do any control work in Washington. The delphinium plant does not reach its best development here, preferring the cooler northern States. The organism also is very sensitive to high temperatures. Moreover, isolations kept on culture media over winter have lost much of their virulence and by the time fresh isolations can be obtained from northern material the weather here has become unfavorable to both host and parasite. Control studies should therefore be made in a region where the disease prevails.

A few suggestions may be made, however. It seems probable that the organism winters-over in the soil since it is rather resistant to cold and drying, and since infection generally appears first on the lower leaves, suggesting the spattering of infected soil by rain. On this theory all diseased material should be gathered and burned. Drenching the soil surrounding the plants with alkaline Bordeaux mixture before growth begins in the spring and later spraying both the soil and the lower surface of early leaves with Bordeaux mixture should prevent infection from this source.

## SUMMARY

The black spot of delphinium is a bacterial disease widespread in the northern part of the United States and is very destructive to choice hybrid varieties.

The bacteria gain entrance to the plant through the water pores and stomata, causing irregular tarry black spots on the leaves and sometimes on stems and flower buds.

The causal bacterium has been isolated and successful infections obtained by spraying with water suspensions of young subcultures from single colonies.

For control of the disease it is suggested that all diseased material be collected and burned and that the surrounding soil and early leaves be sprayed with Bordeaux mixture.

Since the flagella have been demonstrated to be polar, the name of the organism becomes *Bacterium delphinii* (EFS).

PLATE 1<sup>a</sup>

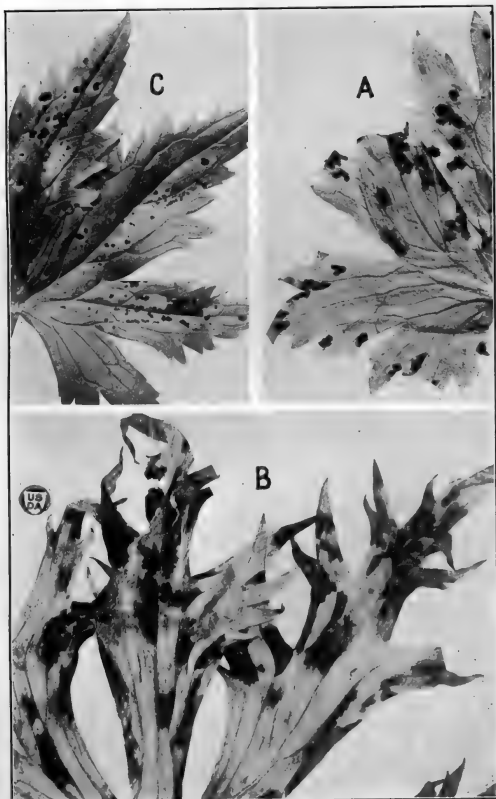
A.—Delphinium leaf from Maine, 1923. Small spots.

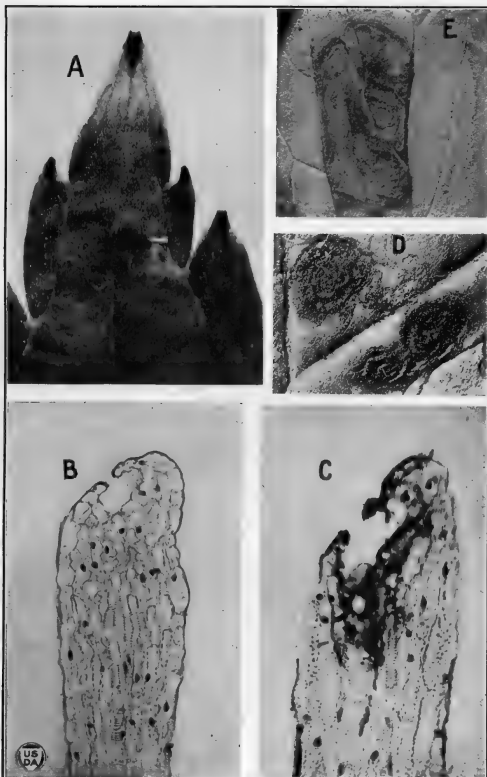
B.—Delphinium leaf from Castine, Me., 1922. Large spots caused by *Bact. delphinii*. Reduced slightly.

C.—Spots on delphinium leaf produced by spraying with water suspension of a subculture of a single colony freshly isolated from a leaf from Maine, in July 1923, 32 days after inoculation.

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<sup>a</sup> All photographs were made by James F. Brewer.





## PLATE 2

A.—Tip of a delphinium leaf showing infected (blackened) water pores. Time, 20 days after inoculation in the hothouse with the organism from Oregon.  $\times 10$ .

B.—Microtome section of a normal water pore. The black spots are nuclei.

C.—Microtome section of an infected water pore.

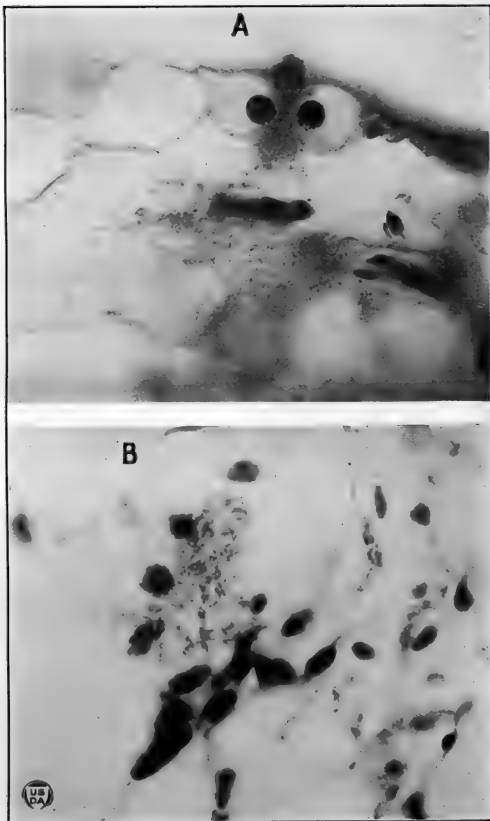
D.—Small leaf spots showing zoning. Photographed from a fresh leaf received from Connecticut.  $\times 4$ .

E.—Large leaf spot showing zoning. Photographed from a pressed leaf from Castine, Me.  $\times 2\frac{1}{2}$ .

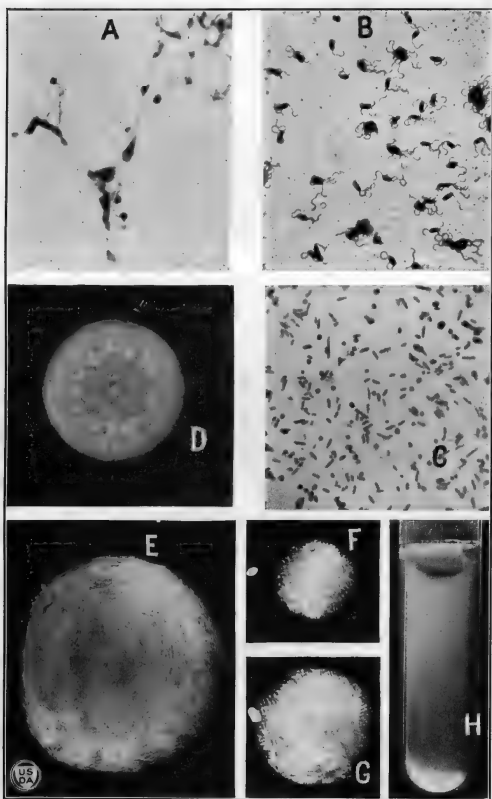
### PLATE 3

A.—Stomatal infection. Microtome section of a leaf spot 48 hours after inoculation by spraying. Bacteria lie on the walls of the cells nearest the stoma, and in and under the stoma.  $\times 1400$ .

B.—Bacteria in the tissues. The large oval bodies are masses of bacteria.  $\times 1400$ .







#### PLATE 4

A.—Hemispherical extrusions on cell walls in the vicinity of the invading bacteria.  $\times 500$ .

B.—*Bacterium delphinii* showing flagella. Stained by Casares-Gil's method.  $\times 940$ .

C.—Rods and spherical bodies from the sediment in the pellicle of bouillon cultures.

D.—Agar plate, colony 4 days old showing ring of opaque areas.  $\times 10$ .

E.—Agar plate, colony 9 days old, by oblique transmitted light.  $\times 10$ .

F.—Agar plate, colony 2 days old, by oblique transmitted light.  $\times 10$ .

G.—Agar plate, colony 3 days old, by oblique transmitted light.  $\times 10$ .

H.—Culture in beef bouillon 4 days old, showing pellicle weighted down in center by a mass of sediment.

# FURTHER OBSERVATIONS ON THE MOLTS OF THE OXBOTS *HYPODERMA BOVIS* DE GEER AND *H. LINEATUM* VILLERS<sup>1</sup>

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## INTRODUCTION

In a paper <sup>2</sup> published by the writer the conclusion was reached that there are five stages or four molts in the larvæ of *Hypoderma lineatum* Villers and probably the same number in *H. bovis* De Geer. These conclusions have been generally accepted as presented by the writer but they have been questioned by some entomologists who hold to the older theory of the occurrence of only three stages or two molts in the case of Oestridæ as well as other Diptera. The purpose of the present paper, therefore, is to present further facts substantiating the claim that there are five larval stages in these two species of *Hypoderma*.

The writer's paper referred to above, setting forth the larval characters for each stage, was brief, and merely the general differences of the cephalopharyngeal skeleton, mouth parts, and spinous armature of each species were given.

The confusion in the larval stages lies in the earlier ones, that is, first, second, and third. The first stage as hatched is well known, as are also the last two stages in the back of the host. Some maintain that the larvæ have no molt from the time they leave the egg and enter the skin of the host until they reach the subdermal tissues of the back approximately eight months later, during this time having increased in length from less than 1 mm. to from 13 to 16 mm.

## COMPARISON OF CHARACTERS OF FIRST THREE LARVAL STAGES IN *HYPODERMA LINEATUM*

For the purpose of comparing some of the more outstanding characters of each of the first three larval stages as described by the writer, three larvæ of each stage taken at random out of a large series of cleared and mounted specimens of *Hypoderma lineatum* were subjected to a microscopic examination, the characters of each instar carefully noted, and measurements made and averaged for the three specimens of each instar. These averages are presented in Table I. The characters noted in the table constitute measurements of the cephalopharyngeal skeleton with mouth hooks, the number of spines in the group immediately below the mouth, the spines on the ventral side of the eighth segment and those on the anal segment, including the flattened or triangular spines on the border of the posterior spiracles. The eighth segment was chosen for special study because the diameter of the larva is near its greatest at this point and the counts and measurements of spines can be made with greater accuracy and ease than on the smaller segments.

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<sup>1</sup> Received for publication March 21, 1924.

<sup>2</sup> LAAKE, E. W.—DISTINGUISHING CHARACTERS OF THE LARVAL STAGES OF THE OXBOT *HYPODERMA BOVIS* AND *HYPODERMA LINEATUM*, WITH DESCRIPTION OF A NEW LARVAL STAGE. *Jour. Agr. Research* 21: 439-457, illus. 1921.

TABLE I.—Dimensions in microns of the cephalopharyngeal skeleton and mouth hooks and the number and dimensions of part of the spinous armature of the first, second, and third larval instars of *Hypoderma lineatum*

Instar	Total length	Expanse of mouth hook from tip to tip	Length of cephalopharyngeal skeleton and mouth hooks	Number of spines in group below mouth	Diameter of eighth segment	Spines on ventral side of eighth segment							
						First row							
						Diameter at base		Length		Distance apart		Number in row	
						Minimum	Maximum	Minimum	Maximum	Minimum	Minimum		
1-----	419	14.1	115.6	36	114	2.6	3.4	5.4	6.3	1.5	3.3	23.0	
2-----	2,312	15.3	117.1	55	731	2.7	3.5	4.5	9.8	17.0	25.6	30.6	
3-----	13,422	16.6	125.5	57	3,462							0.0	

Instar	Spines on ventral side of eighth segment													
	Second row							Third row						
	Diameter at base		Length		Distance apart		Number in row	Diameter at base		Length		Distance apart		Number in row
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum		Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	
1-----	2.0	2.9	4.1	5.5	1.6	3.9	24.0	1.8	2.5	2.9	3.8	3.0	5.0	22.3
2-----	1.9	2.8	4.2	8.2	12.8	31.6	29.6	1.6	2.4	4.5	6.1	10.9	36.3	30.6
3-----							0.0							0.0

Instar	Spines on ventral side of eighth segment							Anal segment						
	Fourth, fifth, sixth and seventh rows							Spines				Length of triangular spines on border of posterior spiracles	Diameter of posterior spiracles	
	Diameter at base		Length		Distance apart		Number in row	Diameter at base		Length				
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum		Minimum	Maximum	Minimum	Maximum			
1-----	1.4	2.9	2.1	4.7	2.3	6.6	21-23	2.4	3.3	4.3	7.3	12.1	5.9	
2-----	1.4	2.3	3.7	6.1	11.6	37.6	29-31	2.7	7.6	4.2	10.5	10.3	9.4	
3-----							0	6.5	22.5	5.7	17.4	5.6	14.1	

The figures in the table reveal marked differences, particularly in the number and the dimensions of the spines of the stages in question. The cephalopharyngeal skeleton and mouth hooks increase in size, as shown by the figures in the table, and the posterior part of the skeleton changes its shape from one stage to the next, spreading out with each molt. Although these differences are not great, there is shown, nevertheless, constant increase in the size of these highly chitinated organs.

If the spines in the group below the mouth are next examined a wide difference in number is found, especially between those of the first and second stages. Since the figures in the table represent averages for each stage, this difference could not be accounted for by individual variation. Among the spines on the ventral side of the eighth segment just as marked a difference in dimensions and number is found. With only one exception, that of the first row, the maximum diameter of the spines at the base is larger in the first than in the second stage. Although the diameter of these spines has decreased slightly from the first to the second stage, the length, without exception, has nearly doubled and the number of spines has likewise increased from 23 to 30.6 for the first row and similarly for all the other rows. Finally, when the spinous armature of the terminal segment is considered, the most striking differences are found. The spines

of the first-stage larva are straight or only slightly curved, with a somewhat blunt point and without a large and heavily chitinized base. The maximum length of the spines is more than twice the maximum diameter at the base. The triangular spines on the border of the posterior spiracles are well pointed, more heavily chitinized, and longer than the other spines on the terminal segment.

In the second stage the spines are very sharply pointed, strongly curved, and only slightly longer than the large, circular, elevated base. The shape of these spines, in every respect, is distinctly different from that found in spines on the first stage. The triangular spines on the border of the spiracles are equal in length or slightly shorter than the longest spines on the terminal segment. They are without a distinct point and shorter than in the first stage. The diameter of the spiracles has increased considerably compared with that of the first stage. In the third stage the spines on the terminal segment again show a striking difference from those of the second stage and the greatly increased dimensions are in a reverse order. The diameter of the enlarged base, nearly three times that of the second stage, is now greater than the length of the spine, which itself has attained a length almost twice that of the second stage. The posterior spiracles have increased their diameter considerably and the triangular spines on the border of the spiracles have decreased in size to almost half that of the preceding stage and are now very blunt, or sometimes slightly knobbed at the point.

Geddoelst,<sup>3</sup> who holds that there are only three stages in the larva of *Hypoderma*, states that when growth takes place within the duration of the same stage it comprises all the soft parts of the organism, but that it does not affect the highly chitinized organs, such as the stigmata, cephalopharyngeal skeleton, and spines, which do not acquire any greater dimensions, except on the occasion of molts. He attributes the greater distance between the spines of the larger (second-stage) larvæ to the growth of the soft parts of the organism, and explains the vastly greater dimensions of the spines on the terminal segment as a continuation of the processes of chitinization.

The greater distance between the spines of the second-stage larvæ could easily be the result of growth of the soft parts of the organism but in such case the number and size of the spines should remain constant. This, however, is not the case. Certainly the increase in the number of spines could not be explained as an extension of the processes of chitinization. By this theory it would seem even more difficult to explain the absence of spines on the body segments of the third-stage larvæ. Koch,<sup>4</sup> who also holds that there are only three larval stages in *Hypoderma*, advances the theory that the spines on the body segments are gradually worn down as the larvæ migrate through the tissues. This would contradict Geddoelst's theory of the continuation of the processes of chitinization as the cause of the enlargement of the spines. Koch's assumption was not correct, however, at least not in so far as the first and second-instar larvæ are concerned, as the spines of the second-stage larvæ, although actually slightly smaller in diameter, with the exception mentioned before, are much longer than in the first stage.

It is not likely that the clearing process in preparing the specimens for slide mounts could be responsible for the disappearance of the spines on all the body segments in the third-stage larvæ and not affect the small spines in the group below the mouth or any of the spines in second-stage larvæ.

Further evidence that there is a molt between the smaller spiny larvæ and the larger spineless larvæ is the fact that occasionally a larva is found that possesses one or sometimes several small groups of highly chitinized spines on

<sup>3</sup> GEDDOELST, L.—LE TRIMORPHISME LARVAIRE DES OESTRIDÉS. *Compt. Rend. Soc. Biol. [Paris]* 86: 501-504. 1922.

<sup>4</sup> KOCH, T. P.—OM OKSEBREMSSEN HYPODERMA BOVIS. *Maanedsskr. Dyrlaeger* 15: 129-159. 1903

some one of the body segments. These spines, although similar in shape and size to those in rows on the earlier stages, are not placed in regular order as they appear in either the first or second stage. The occurrence of a small group of irregularly arranged spines on the otherwise spineless body segments of the third stage can be explained as an occasional individual variation, and clearly indicates their presence as the result of a molt, as spines so arranged are never present in the first or second instars.

Although the characters as set forth above should be sufficient to show clearly that there are at least three distinct stages of *Hypoderma lineatum* in the course of the migration of the larvæ from the point of entrance to the place of encystment on the back, where two subsequent larval molts take place, it has been the author's good fortune to obtain two larvæ of *H. lineatum* during the process of molting from the second to the third stage, thereby definitely establishing this fact for one of these molts, at least. These larvæ, both approximately 13 mm. in length, were taken, one from the gullet and one from the diaphragm of two different animals slaughtered in a packing house at Dallas, Tex.

The specimen taken from the gullet, although almost completely free from its cast-off second-stage exuvium, was still possessed of part of it. The newly molted specimen, a third-stage larva, is free from spines on the body segments while the remaining part of the exuvium extending back nearly as far as the third segment of the larva, and plainly showing the torn borders, is covered with the usual second-stage armature. In the case of the larva taken from the diaphragm, the entire and unbroken second-stage exuvium was present and was easily removed from the smooth larva. This newly molted third-stage specimen, spineless on all the body segments, together with the cast-off second-stage exuvium possessed of the entire spinous armature of that stage, was cleared, mounted, and preserved.

The rarity of finding a larva in the process of molting is no doubt due to the very delicate structure of the exuvium and the extreme difficulty in removing the larva from the connective tissue of the host without losing the skin. In the case of the first molt, which probably takes place soon after the larva gains entrance to the host, when it is extremely small, it is almost hopeless to obtain a specimen at the instant of molting from the first to the second stage. In fact, no first-instar larvæ have ever been found after having left the point of entrance.

#### THE SECOND-STAGE LARVA OF *HYPODERMA BOVIS*

The second-stage larva of *Hypoderma bovis* was unknown in 1921, at the time the writer's preceding paper was published, but its existence was considered probable and deemed necessary to fill the gap between the first and third stages which are so similar to those stages in *H. lineatum*. Since then Phibbs<sup>5</sup> of Ireland has collected and described this stage. At about the same time Wells and Brundrett, of the Bureau of Entomology, who conducted investigations in the State of New York, made a number of collections of second-stage larvæ of *H. bovis*, all of which, with the exception of a single specimen from the gullet, were taken from the neural canal of slaughtered cattle.

The characters of the second-stage larva of *Hypoderma bovis* are remarkably similar to those of *H. lineatum* in the same stage, except, of course, the difference, as pointed out before, in the mouth hooks and in the anterior end of the cephalopharyngeal skeleton. The arrangement of the spinous armature of the head segments, the transverse rows of spines on the body segments, with the heavier spines in the anterior rows of each segment, and the spines, with broad bases, on the anal segment are all almost identical in appearance with those of *H. lineatum* in the same stage.

<sup>5</sup> PHIBBS, G.—THE LARVAL MOUTH-HOOKS OF *HYPODERMA*. Irish Nat. 31: 25-30, illus. 1922.

# FLAVOBACTERIUM SUAVEOLENS, A NEW SPECIES OF AROMATIC BACILLUS ISOLATED FROM DAIRY WASTES<sup>1</sup>

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## INTRODUCTION

In a large group of organisms isolated from dairy wastes were found several which produced an aromatic odor from all of the mediums containing protein. The odor is sweet-scented, very pleasing during the first two days' incubation at 22° C. but in four to six days it changes to a decided cheesy odor which persists for two months. At 37° the cheesy odor develops in two days. During the three years these organisms have been under observation, this property of aroma production has always been manifested in subcultures, apparently undiminished in intensity.

As the aroma-producing property seemed to be associated with the nitrogenous organic matter in the medium, it was anticipated that the organism was an active proteolyzer. Inoculated into a 1½ per cent gelatin with an initial P<sub>H</sub> of 7.3 and held at 22° C. for six days, the amino and ammoniacal nitrogen increased from 50 to 950 p. p. m. Inoculated into skim milk and kept under similar conditions, the amino and ammoniacal nitrogen increased from 30 to 300 p. p. m.

## MORPHOLOGY

Smears from plain agar (12 hours—7 days) are always Gram-negative. Milk powder agar cultures, 12 to 16 hours old, are Gram-positive and become Gram-negative on further incubation; spores were not observed when tested by spore stains (24-hour to 7-day old agar culture) or by exposure to temperatures of 60° to 80° C.; growth was not obtained after heating either a young or old culture at 60° for 10 minutes or at 80° for 5 minutes; size varies from 1.0μ to 1.2μ by 0.6μ to 0.8μ; rods have rounded ends; actively motile, position of flagella peritrichous.

## CULTURAL CHARACTERISTICS

Records are for 22° C. for 48 hours unless otherwise stated. The P<sub>H</sub> of media employed ranged from 6.5 to 7.5

**NUTRIENT-AGAR SLANT.**—Growth is moderate, filiform, flat, glistening, opaque, of butyrous consistency and produces an aromatic odor; pigment is yellow—510.033 shade 2<sup>1</sup>; pigment is insoluble in water, methyl, ethyl, and isoamyl alcohols, benzene, carbon tetrachlorid, and xylol; readily soluble in acetone and slowly soluble in ether and chloroform; in 6 days growth is oleaginous.

**NUTRIENT-AGAR PLATES.**—Colonies small, growth similar to slant; confluence marked in seven days.

**MILK POWDER AGAR SLANT.**—Similar to plain agar but growth more abundant and color deeper—420.042 shade 3<sup>1</sup>; proteolysis distinct.

**NUTRIENT BROTH.**—Moderate uniform clouding, scanty sediment; no surface growth; an aromatic odor which changes to cheesy in four days.

**GELATIN STAB.**—A stratiform liquefaction is visible in one-half day; gelatin completely liquefied in six days; medium brownish and white precipitate settles at bottom by sixth day.

**POTATO SLANT.**—Abundant growth, color more intense than on nutrient or milk powder agar—330.051 shade 3<sup>1</sup>.

<sup>1</sup> Received for publication Jan. 26, 1924.

## PHYSIOLOGICAL REACTIONS

The carbohydrates are weakly attacked. In seven days a slight acidity but no gas can be detected in dextrose, sucrose and glycerin broths<sup>2</sup>; neither acid nor gas in lactose or starch; in a 0.4 per cent caseinate solution plus 1 per cent lactose, the  $P_H$  changed from 7.0 to 6.9 in three to seven days at 22° C.; no diastatic action was evident on starch agar.

MILK.—The organism proteolyzes milk rapidly; the reaction remains unchanged or becomes slightly alkaline; no curd can be detected even by heating but on long incubation a powdery amorphous precipitate develops; litmus reduces in seven days. In whole milk the cream layer is unaffected; tubes of whole milk incubated at 37° C. or at 22° for two months did not have the cream layer visibly altered; the milk beneath the cream layer is digested nearly as rapidly as in skim-milk tubes; the odor remains cheesy, but is not considered putrid.

NO<sub>3</sub> BROTH.—Nitrates are not reduced in 10 days at 22° C.

INDOL PRODUCTION.<sup>3</sup>—Indol present in seven days at 22° C.

H<sub>2</sub>S PRODUCTION.<sup>4</sup>—H<sub>2</sub>S is produced in three to seven days at 22° C.

RELATION TO OXYGEN.—Grows best aerobically.

Omelianski (2)<sup>5</sup> in a recent article quite thoroughly reviews the literature on aroma-producing microorganisms. Several species have been described which bear close resemblance to the organisms under consideration. Among them are *Bacillus praepollens* Maaszen (1) and *Flavobacterium aromaticum* Pammel (3). These, however, differ in several characters. *Bacillus praepollens* of Maaszen is nonmotile, does not produce indol and produces black granules in broth and in gelatin. Dr. Pammel's *F. aromaticum* is indol-negative and produces gas from dextrose and sucrose.

A thorough search of the literature has not revealed a previous description which adequately fits this organism. In the absence of a previous description, the Latin word *suaveolens*, meaning sweet smelling or fragrant, is suggested as the species name. According to Bergey's "Manual of Determinative Bacteriology" (4) it belongs in the genus *Flavobacterium*—hence the name *Flavobacterium suaveolens*.

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<sup>2</sup> Medium employed:

1 per cent Andrade's indicator  
1 per cent peptone  
0.5 per cent NaCl  
0.2 per cent K<sub>2</sub>HPO<sub>4</sub>  
1 per cent carbohydrate.

<sup>3</sup> This was tested according to Malone and Gore's cotton plug test.

<sup>4</sup> The method of the Society of American Bacteriologists, 1920, was used.

<sup>5</sup> Reference is made by number (italic) to "Literature cited," p. 276.



# SOME RELATIONS BETWEEN THE GROWTH AND COMPOSITION OF YOUNG ORANGE TREES AND THE CONCENTRATION OF THE NUTRIENT SOLUTION EMPLOYED<sup>1</sup>

By H. S. REED, *Professor of Plant Physiology*, and A. R. C. HAAS, *Assistant Professor of Plant Physiology, College of Agriculture, University of California*

## INTRODUCTION

The present paper is devoted to a discussion of the effects of different concentrations of the nutrient medium upon young Valencia orange trees (*Citrus sinensis*) and seedlings of the African sour orange (*Citrus aurantium*).

## THE RELATIVE CONSTANCY OF THE CONCENTRATION AND REACTION OF LEAF SAP

The data on this point were obtained from young Valencia orange trees in cultures like those which have been previously described by the writers (3)<sup>2</sup>. The nutrient solutions applied were either Hoagland's solution (3) or modifications thereof. None were strongly toxic although growth was greatly retarded in certain series. Their osmotic concentrations as determined from the freezing-point depression ranged from 0.531 to 1.568 atmospheres, and their  $P_H$  values ranged from 5.2 to 7.5. The plan of the experiments made it impracticable to determine the reaction of the nutrient solution after it was added to the sand cultures, but the reaction of the first 100 cc. of percolate collected after adding distilled water to the surface of the cultures was determined. The first drops collected had the highest  $P_H$  values. All percolates had higher  $P_H$  values than the nutrient solutions which had been applied. In the case of solutions which contained 1,000 parts per million of sodium bicarbonate (series 30-35 and 36-41) the  $P_H$  of the percolates was approximately 8.3. The concentration of OH ions in the percolate from soil cultures which had previously received sodium chlorid solution was equally high. The replacement phenomena involved in such cases have been discussed by Cummins and Kelley (1).

The data (Table I) show that the  $P_H$  and concentration of the leaf sap from trees in the various cultures were remarkably uniform. The small differences found seem to bear no relation to the  $P_H$  or to the concentration of the nutrient solution nor to the  $P_H$  of the percolates. It appears that the absorption and metabolism of the orange trees produce a fairly constant reaction and concentration of the leaf sap. These results are not necessarily contradictory to those obtained by Haas (2) with the sap of *Melilotus alba*, where it was found that a gradient may exist in the plant. It is essential, however, that other plants be examined because any hypothesis based upon differences of acidity of the plant sap and the external medium must account not only for the accumulation of inorganic elements within the roots, but also for their movement to other portions of the plant in which different acidities may prevail. Moreover, the conditions of solubility in the root system may not be the same as those in other parts of the plant.

<sup>1</sup> Received for publication Nov. 26, 1923, Paper No. 116, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, Calif.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 284.

## THE EFFECT OF CONCENTRATION UPON THE GROWTH AND COMPOSITION OF ORANGE SEEDLINGS

A series of water cultures was conducted in a glass house for a period of 122 days to study the effects of six concentrations of nutrient solution upon seedlings. The concentration of the nutrient solutions ranged from 364 to 3635 parts per million. The solution used as a standard had the following composition expressed in parts per million:

Na	K	Ca	Mg	Mn	Fe	Cl	NO <sub>3</sub>	SO <sub>4</sub>	PO <sub>4</sub>	Total
7	185	158	54	Trace.	Trace.	10	718	216	105	1,454

If we represent the total concentration of the standard as C, we may designate the series employed as .25 C, .50 C, 1.0 C, 1.5 C, 2.0 C, and 2.5 C. Eighteen 1-liter glass jars each containing three African sour orange seedlings (*Citrus aurantium*) were used for each of the six concentrations. Each week the solutions in the jars were changed and the water loss measured. At the end of the 122-day period the 54 seedlings used in each set of cultures were removed, weighed, and dried at a temperature between 60° and 70° C. The tops had attained a height of about 15 cm. The leaves, stems, and roots were kept separate for further determinations.

TABLE I.—Relations between the concentration and reaction of nutrient solution and of the leaf sap of trees to which the nutrient solution was applied

Series	Nutrient solution		Percolate	Leaf sap	
	Osmotic pressure	P <sub>H</sub>	P <sub>H</sub> of first portion of percolate	P <sub>H</sub>	Osmotic pressure
SAND CULTURES					
1-5.....	Atm.				Atm.
6-11.....	0.728	5.2	5.9-7.2	6.0	20.8
12-17.....	1.459	5.2	6.6-8.0	5.88	22.59
18-23.....	1.568	5.2	5.4-7.8	5.88	21.26
24-29.....	1.001	5.2	6.4-8.0	5.85	23.10
30-35.....	1.218	5.2	5.8-7.35	5.87	20.45
36-41.....	1.278	7.55	8.3+c	5.95	21.47
42-46.....	1.447	7.45	8.3+c	5.80	21.17
47-51.....	0.708	5.2	7.0-7.4	5.95	22.53
	0.531	5.2	6.0-7.2	5.77	21.89
SOIL CULTURES					
84-88.....		(a)		5.80	21.03
89-92.....		(b)	(c)	5.82	20.32

<sup>a</sup> Distilled water only was added.

<sup>b</sup> Distilled water containing 1,500 parts per million NaCl was added.

<sup>c</sup> Sodium carbonate present.

The data on the weight of the plants and of their ash are given in Table II, and show that the best growth of seedlings was made in 1.5 C, a solution which had an initial concentration of 2,181 parts per million. The plants grown in .25 C and .50 C were inferior to those in the other concentrations. The differences in both green and dry weight of tops were greater than for the corresponding weights of roots; in fact, the latter were rather uniform. By way of comparison, it may be stated that 54 seedlings of the size of those used to start these cultures had a green weight of 21.4 gm. and a dry weight of 3.52 gm.

The data on transpiration per unit of dry weight of tops show considerably more variation than when computed per unit of dry weight of the entire plant. The

ratio of water transpired to unit of dry weight was least in case of the largest plants (as is usually the case), and greatest in the case of the smallest plants.

Table III shows data obtained from the analysis of the leaves and stems of the seedlings just described. The leaves showed an increasing percentage of ash, with increasing concentration of culture solution. The percentage of calcium in the ash of the leaves showed a steady decline in the lowest four concentrations, while the percentages of potassium showed a rise in the higher concentrations. On the other hand, the percentage of sulphate and phosphate was lowest in the cultures receiving the three highest concentrations.

The ash of the stems increased with increasing concentrations of culture solution, but the percentage of ions in the ash showed no pronounced changes corresponding with increasing concentrations of culture solution. The last column in Table III shows the composition of entire seedlings from the same source and of the same age as those used in installing the cultures. Attention should be paid to their content of sodium, potassium, and phosphoric acid. Computation of the actual amounts of sodium and phosphoric acid in the dry matter of the seedlings shows that the stems have lost rather than gained in these ions during the course of growth in 122 days.

TABLE II.—*African sour orange seedlings in various concentrations of nutrient solution. (Each lot consisted of 54 seedlings having an initial green weight of 21.4 gm. and dry weight of 3.52 gm.)*

		CONCENTRATION OF NUTRIENT SOLUTION					
		.25 C	.50 C	1.0 C	1.5 C	2.0 C	2.5 C
		Grams	Grams	Grams	Grams	Grams	Grams
Green weight.....	Tops.....	37	44	66	92	84	78
	Roots.....	45	39	51	58	52	55
	Entire plants.....	82	83	117	150	136	133
	Leaves.....	6.84	8.76	13.39	19.09	16.80	14.07
Dry weight.....	Stems.....	4.45	4.03	5.80	8.10	6.36	6.50
	Roots.....	8.91	7.79	10.47	11.87	10.60	11.00
	Tops.....	11.29	12.79	19.19	27.19	23.16	20.57
	Entire plants.....	20.20	20.58	29.66	39.06	33.76	31.57
Weight of ash.....	Tops.....	0.869	1.072	1.769	2.629	2.656	2.553
	Roots.....	0.756	0.696	1.065	1.224	1.228	1.216
	Entire plants.....	1.625	1.768	2.814	3.853	3.884	3.769
Water transpired per gram of dry weight.	Tops.....	893	826	673	564	603	633
	Entire plant.....	499	514	435	393	414	412

TABLE III.—*Composition of the ash of the leaves and stems of orange seedlings from solutions of various concentrations*

Concentration of nutrient solutions	Leaves						Stem						Entire seedlings from germ-inating box
	0.25 C	0.50 C	1.0 C	1.5 C	2.0 C	2.5 C	0.25 C	0.50 C	1.0 C	1.5 C	2.0 C	2.5 C	
Percentage of ash in the dry matter....	8.50	8.68	9.59	10.24	12.11	13.16	6.49	7.73	8.19	8.31	9.77	10.79	5.58
Percentage of water soluble inorganic matter.....	41.03	46.66	51.39	51.99	60.18	57.68	59.19	61.11	62.09	66.67	67.49	69.07	-----
Constituents as a percentage of the ash:													
Na.....	3.05	3.88	5.10	6.31	7.70	6.60	4.54	6.17	5.07	6.11	6.68	-----	13.29
K.....	15.39	20.77	23.35	23.66	28.02	26.87	25.94	27.75	27.93	30.11	29.07	-----	27.73
Ca.....	17.62	14.55	11.13	7.46	7.77	8.85	13.90	13.52	13.01	11.61	9.43	10.96	6.32
Mg.....	3.12	2.25	3.90	-----	-----	-----	3.40	3.92	2.88	3.08	4.50	3.91	3.51
Cl.....	.46	.71	.78	.53	1.10	.37	1.91	2.31	2.74	1.90	2.90	1.31	1.07
SO <sub>4</sub> .....	7.66	6.31	6.24	5.30	4.97	5.14	6.31	5.88	6.23	4.74	5.10	5.41	5.45
PO <sub>4</sub> .....	8.36	7.75	8.41	5.66	5.31	5.66	13.59	10.40	10.69	11.16	9.20	9.21	26.97

# THE EFFECT OF CONCENTRATION UPON THE GROWTH AND COMPOSITION OF YOUNG VALENCIA ORANGE TREES IN SAND CULTURES

In experiments on this question we employed 1-year-old orange trees growing in large galvanized iron cans containing silica sand. The cans were provided with means for adding nutrient solutions and collecting drainage water according to methods previously described (3). The nutrient solutions employed were based on that used in the water cultures described in an earlier paragraph, and were .25 C, .50 C, and 2.0 C. They had total concentrations, therefore, of 364, 727, and 2908 parts per million. It is not feasible to maintain rigidly a given concentration of nutrient solution in sand cultures, but by frequent renewals it was possible to maintain the approximate concentrations. The cultures which had the .25 C solution were renewed at intervals of 7 to 10 days, the others were renewed at intervals of 14 to 21 days. The trees were planted in the sand cultures April 5, 1921, and grew for 21 months.

The leaves of the trees receiving .25 C nutrient solution were yellow-green; those receiving .5 C solution were dark green but not nearly so deep a green as the leaves of trees receiving 2.0 C solution. The rootlets in the .25 C solution when in the fresh condition appeared to be finer than in the highest concentration.

TABLE IV.—*Data on the growth of young Valencia orange trees in sand cultures to which nutrient solutions of various concentrations were added. (Each lot consisted of 6 trees, and figures represent averages.)*

	Concentration of nutrient solution		
	0.25 C	0.50 C	2.0 C
Number of leaves per tree.....	931	1,162	1,351
Number of yellow leaves per tree.....	48	57	---
Green weight (in grams) {	Leaves.....	653	748
	Shoots.....	290	342
	Trunk.....	507	484
Dry weight (in grams) {	Leaves.....	177	227
	Shoots.....	125	160
	Trunk.....	261	313
	Root.....	243	324
	Rootlets.....	300	376
	Total.....	1,106	1,400
Volume of liquids added to cultures (in liters) {	Nutrient solution.....	253	173
	Distilled water.....	391	471
Volume of drainage water recovered (in liters).....	224	170	171
Water transpired by trees (in liters).....	420	474	452
Ratio of transpiration to dry weight {	Entire tree.....	380	339
	Leaves.....	2,373	2,090
			1,656

Table IV gives the dry weight of the different portions of the trees, and also the green weights of the tops of the trees in the .5 C and the 2.0 C cultures. The green and dry weight of the leaves and shoots increased with increasing concentration of the culture solution. The diameter and appearance of the trunk and root of the different trees were quite uniform at the time of planting. However, there must have been some initial differences in weight of the tree portions when planted, hence differences in the green or dry weights of the trunks and roots will not be emphasized. Trees receiving .5 C solution produced the largest dry weight of rootlets. The total dry weights of trees receiving .5 C or 2.0 C solution were practically identical, but the average dry weight of trees receiving .25 C solution was less than the others. The water requirement per tree in each series showed slight reduction with increasing concentration

of culture solution, but the ratio of the total dry weight of leaves and shoots to that of rootlets increased with increasing concentrations of culture solution. The increased nitrate content of the more concentrated solutions may play an important part in determining the ratio of tops to roots.

Photographs were taken of the trees just prior to their removal from the containers, nearly 21 months after the trees were planted. Plate 1, A, B, and C, shows the condition of the trees receiving different solutions, and D shows their root systems.

The per cent of ash (Table V) in the various portions of the trees is greatest in those trees receiving the double strength solution. The percentages of ash in the rootlets show the largest differences, the per cent of ash in the rootlets of the trees receiving double strength solution being about twice that of the rootlets receiving quarter or half strength solutions. The composition of the ash of the three series of trees was not materially different except in the case of  $\text{PO}_4$ . The percentages of each cation in the leaves, shoots, trunk, and root are approximately constant for the different concentrations of culture solution. The percentage of sodium in the ash of the rootlets increases with increasing concentration of the culture solution, while that of potassium is approximately constant at the two lower concentrations of culture solution, but is higher in the ash of the rootlets at the highest concentration.

Table V shows that in general there is very little change in percentage composition with increasing concentration of culture solution. The trees apparently utilized the increase of nutrient ions in large measure for increases in size instead of producing a given amount of growth and accumulating large concentrations of certain ions, as frequently happens when trees are grown in unfavorable culture solutions.

#### THE EFFECT OF CONCENTRATION UPON THE SOLUBILITY OF INORGANIC CONSTITUENTS OF THE DRY MATTER OF THE TREES

Table V contains data showing the soluble fraction of the constituents determined in the dry matter of all portions of these trees. The fraction dissolved in water was greatest in the rootlets, and least in the trunk. There was a tendency toward increased solubility in the ash as the concentration of the nutrient solutions was increased. Taking a general view of the data presented in Table V, it appears that an increased concentration of salts in the nutrient solution results not only in an increased concentration of ions in the ash of the trees which received them, but in an increase of the fraction which is soluble in water. The case of calcium forms a conspicuous exception to the statement just made. This accumulation of surplus materials in a soluble state should set aside certain assumptions on the principles of absorption by plants which postulate insolubility instead of solubility (6). Moreover, the discovery of this condition in orange trees explains certain effects which have been observed in soils of high salt content. We should especially like to emphasize the importance of the calcium relation. Previous papers by the writers (4 and 5) have contained data on the harmful effects of sodium salts and on the ameliorating effects of calcium salts upon orange trees. Now, if the solubility relations are maintained as concentrations increase, it is apparent that as the concentration of the harmful sodium salts increases in the trees their relative solubility increases, while as the concentration of the beneficial calcium ion increases its relative solubility decreases, except in the leaves. If this be the case, we are then in a better position to understand the results often observed when trees grow in soils of high salt content.

TABLE V.—Composition of the ash and water solubility of inorganic constituents of dry matter of young orange trees grown in sand cultures

	Leaves						Shoots					
	0.25C		0.50C		2.0C		0.25C		0.50C		2.0C	
	Per cent of ash	Per cent H <sub>2</sub> O soluble	Per cent of ash	Per cent H <sub>2</sub> O soluble	Per cent of ash	Per cent H <sub>2</sub> O soluble	Per cent of ash	Per cent H <sub>2</sub> O soluble	Per cent of ash	Per cent H <sub>2</sub> O soluble	Per cent of ash	Per cent H <sub>2</sub> O soluble
Ash as percentage of dry matter.....	13.73	-----	13.49	-----	14.34	-----	4.48	-----	4.30	-----	6.30	-----
Inorganic constituents of dry matter.....	-----	47.47	-----	54.47	-----	58.81	-----	39.48	-----	39.62	-----	38.94
Na.....	4.57	85.68	4.06	88.10	4.58	95.74	3.97	69.87	3.86	84.16	3.29	81.02
K.....	18.05	86.21	18.20	95.43	18.23	97.93	15.10	89.49	15.59	91.08	14.89	91.79
Ca.....	20.37	23.21	19.81	27.33	20.88	34.65	21.99	11.60	21.85	9.17	22.95	9.02
Mg.....	2.15	60.74	1.97	75.24	1.85	79.62	3.15	55.63	3.17	54.35	2.40	56.95
Cl.....	0.26	100.00	0.26	100.00	0.30	100.00	0.37	100.00	0.40	100.00	0.30	100.00
SO <sub>4</sub> .....	3.94	65.53	4.34	71.58	4.39	68.89	3.43	61.92	3.53	66.04	4.86	70.16
PO <sub>4</sub> .....	2.84	56.43	3.41	62.09	4.70	69.58	4.57	57.82	5.37	63.91	7.77	84.24

	Trunk						Root					
	0.25C		0.50C		2.0C		0.25C		0.50C		2.0C	
	Per cent of ash	Per cent H <sub>2</sub> O soluble	Per cent of ash	Per cent H <sub>2</sub> O soluble	Per cent of ash	Per cent H <sub>2</sub> O soluble	Per cent of ash	Per cent H <sub>2</sub> O soluble	Per cent of ash	Per cent H <sub>2</sub> O soluble	Per cent of ash	Per cent H <sub>2</sub> O soluble
Ash as percentage of dry matter.....	2.89	-----	2.38	-----	3.21	-----	2.42	-----	2.56	-----	2.91	-----
Inorganic constituents of dry matter.....	-----	30.12	-----	34.93	-----	35.02	-----	37.16	-----	35.09	-----	42.96
Na.....	3.53	68.62	4.44	72.35	3.42	83.99	2.92	96.39	3.29	100.00	3.71	100.00
K.....	12.86	87.36	15.20	87.59	13.97	93.10	14.48	92.22	13.68	92.13	15.59	94.51
Ca.....	19.89	7.24	20.68	6.79	22.70	6.51	18.48	6.11	16.48	5.46	17.73	6.56
Mg.....	1.61	44.92	1.91	40.50	1.68	57.28	1.95	32.64	1.98	63.19	2.35	45.74
Cl.....	0.46	100.00	0.47	100.00	0.39	100.00	0.50	100.00	0.43	100.00	0.38	100.00
SO <sub>4</sub> .....	2.82	27.78	2.89	39.94	3.75	43.36	2.02	35.81	2.62	35.47	2.68	41.16
PO <sub>4</sub> .....	3.94	66.19	5.89	72.30	6.82	80.55	6.85	57.53	7.92	55.04	8.15	70.90

	(Silica-free) Rootlets					
	0.25C		0.50C		2.0C	
	Per cent of ash	Per cent H <sub>2</sub> O soluble	Per cent of ash	Per cent H <sub>2</sub> O soluble	Per cent of ash	Per cent H <sub>2</sub> O soluble
Ash as percentage of dry matter.....	5.03	-----	4.91	-----	9.14	-----
Inorganic constituents of dry matter.....	-----	64.99	-----	65.31	-----	69.77
Na.....	4.06	100.00	5.54	98.97	7.09	98.78
K.....	19.66	93.51	18.19	94.69	28.33	95.89
Ca.....	7.52	35.72	7.11	32.59	7.80	21.39
Mg.....	8.05	71.14	7.79	68.76	3.71	65.90
Cl.....	1.65	100.00	1.85	100.00	1.57	100.00
SO <sub>4</sub> .....	8.08	57.86	10.14	70.32	12.52	86.40
PO <sub>4</sub> .....	7.34	42.92	8.83	39.59	12.05	36.62

• These do not include organic S or P.

THE ASH OF TREES GROWN IN SOIL

The foregoing pages have discussed the composition of trees grown in sand cultures to which nutrient solutions were added. We shall present similar data upon trees grown in soil (Table VI). The results are also given for the water solubility of the inorganic constituents of the dry matter. The dry matter was placed on a quantitative filter paper in a funnel and was washed with distilled water until approximately 600–800 cc. of filtrate were obtained. After each application of distilled water to the dry matter, the distilled water was not renewed until the filtrate ceased dropping from the funnel. The filtrate was evaporated to dryness in a porcelain dish and ignited at low heat.

The ash obtained from the filtrate after leaching the dry matter of the various parts to the trees decreases as we pass from the leaves to the rootlets, where the solubility is approximately that of the shoots. The ash obtained from the filtrate of leached leaves constituted 57 per cent of the total ash found in the entire dry matter of the leaves. There is considerable water-soluble sodium in the leaves, but it remains fairly constant at a lower value in other portions of the trees. However, the actual amounts of sodium present in these trees are relatively small.

The solubility of calcium deserves particular attention, only 45.82 per cent being soluble in the leaves, 15.03 per cent in the shoots, while the trunk, root, and rootlets contained only 7 to 9.5 per cent soluble calcium.

In comparing these trees with those from the sand cultures (Table V) one sees that the percentage of potassium was lower in the trees from the soil, while that of calcium was higher. The solubility of sodium was less in the ash of the trees from the soil and that of calcium was slightly higher. With the exception of the rootlets, the sulphates and phosphates were more soluble in the trees from the soil than in those from the sand cultures.

TABLE VI.—*Data on the composition of orange trees grown in soil; analysis of the ash and the water-soluble fractions of the dry matter*

	Leaves		Shoots		Trunk		Root		Rootlets	
	Per-centage	Solu-bility	Per-centage	Solu-bility	Per-centage	Solu-bility	Per-centage	Solu-bility	Per-centage	Solu-bility
Nitrogen in the dry matter.....	1.94	-----	0.85	-----	0.52	-----	0.51	-----	1.19	-----
Ash in the dry matter.....	13.96	57.02	5.90	39.10	2.34	35.71	2.37	27.42	8.06	40.66
Ash constituents:										
Na.....	2.06	83.30	3.71	62.82	5.42	57.24	7.59	66.74	2.91	64.87
K.....	8.43	99.24	9.52	97.24	12.34	96.93	8.01	92.94	13.53	95.18
Ca.....	28.16	45.82	28.01	15.03	23.51	8.69	19.60	7.18	18.18	9.39
Mg.....	2.51	85.84	2.90	67.16	2.34	48.59	1.69	36.45	2.70	67.92
Cl.....	0.28	100.00	0.41	100.00	0.74	100.00	1.43	100.00	4.67	99.20
SO <sub>4</sub> .....	4.64	81.73	4.16	73.88	4.43	55.22	4.54	47.25	11.30	75.97
PO <sub>4</sub> .....	3.29	94.58	6.50	90.09	7.71	76.96	9.32	63.64	5.18	58.55

SUMMARY

- (1) The sap expressed from leaves of orange trees showed remarkable constancy in the concentration of solutes and in hydrogen-ion concentration, although grown in nutrient solutions of differing composition.
- (2) Orange seedlings grown in water cultures, ranging in concentration from 364 to 3,635 parts per million of solutes, indicated that the most favorable con-

centration was near 2,200 parts per million. This was also the concentration at which the ratio of transpiration to dry weight was lowest.

(3) Young orange trees grown in sand cultures supplied with nutrient solutions containing 364, 727, and 2,908 parts per million of solutes made practically the same amount of growth in each of the last two concentrations mentioned. The percentage of ash in the dry matter was greatest in the case of trees which received the most concentrated solution. The composition of the ash showed less tendency to vary than in the case of seedlings grown in water cultures, though there were some well-defined differences in the ash of rootlets.

(4) The water-soluble inorganic constituents of the dry matter were usually greater in the case of trees which received the more concentrated nutrient solutions. Certain exceptions were found in the case of calcium. There seems to be no evidence in support of the assumption that the absorbed materials are converted into insoluble compounds as rapidly as they accumulate in the plant nor that absorption necessarily depends on the precipitation of ions within the plant. The nature of injuries which orange trees often exhibit when grown in saline soil may be related to the fact that the percentage of soluble sodium compounds appears to rise faster than the percentage of soluble calcium compounds.

(5) The solubility of the inorganic constituents of the dry matter varied considerably in various parts of the tree. The highest percentage of soluble materials was found in the dry matter of the leaves, and the next highest, with the exception of phosphates, in the rootlets. The lowest percentage of soluble materials usually occurred in the trunk and root. In other words, the greatest proportion of soluble materials was found in the parts of the tree in which the most active metabolism occurs.

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### PLATE 1

- A.—Orange trees in sand cultures which had received .25 C nutrient solution.
- B.—Orange trees in sand cultures which had received .50 C nutrient solution.
- C.—Orange trees in sand cultures which had received 2.0 C nutrient solution.
- D.—The root systems of orange trees from sand cultures: No. 93 received .25 C solution; No. 99 received .50 C solution; and No. 110 received 2.0 C solution.



# THE GERMINATION OF COTTONSEED<sup>1</sup>

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## INTRODUCTION

During 1921 and 1922, G. W. R. Davidson, of the Office of Crop Acclimatization and Adaptation Investigations of this Department, called to our attention the fact that our germination tests of cottonseed, especially of seed from Texas, gave very variable and often unaccountably low results. At about the same time the Texas Agricultural Experiment Station discussed with the Seed Testing Laboratory the difficulties of securing reliable results in making germination tests of cottonseed. We have attempted to analyze the situation and to locate the difficulties. Although all cottonseed are sensitive to conditions of germination, a good sample will germinate promptly and vigorously under a comparatively wide range of moisture and temperature conditions. Occasional samples, due to heating in storage or to other factors, contain many seeds with dead or weak embryos; such samples will not germinate vigorously under any conditions. On the other hand, a great many samples of cottonseed, when germinated by the standard method,<sup>3</sup> are conspicuous because of excessive mold and because of the "damping off" of the sprouts. This condition is most frequent in Texas seed of certain varieties.

## VARIATIONS IN GERMINATION RESULTS

One of the great difficulties in germinating cottonseed from Texas has been the great variation in the results obtained from different tests of the same sample. Fourteen separate tests of a single sample by the standard method, all put in the germinator at the same time, gave results ranging from 34 per cent to 58 per cent; with another sample, the results varied from 14 per cent to 46 per cent. These same samples, when tested in small boxes of soil in the germinator, produced over 90 per cent of seedlings which were uniformly vigorous and fully as strong as those from samples which did not mold by the standard method. Great variations in the results of germination tests occur also when various methods are used in making the tests, as is illustrated by the average germination of 12 samples, all of one variety and received at the same time from a large seed firm. The average germination of the 12 samples was as follows: Standard method, 65.6 per cent; in soil in laboratory, 84.1 per cent; in sphagnum moss, 85.1 per cent; in soil in a cool greenhouse, 72.9 per cent; in soil in a warm greenhouse, 92.3 per cent.

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<sup>2</sup> The writers are indebted to W. L. Goss, G. W. R. Davidson, H. C. McNamara, W. W. Ballard, and B. M. King, of this Bureau, and A. H. Leidigh of the Texas Agricultural Experiment Station for aid in the field tests and for helpful suggestions during the progress of the work.

<sup>3</sup> As given in the "Rules for seed testing," adopted by the Association of Official Seed Analysts of North America, the dry seeds are put between folds of moist cotton flannel or of moist absorbent paper toweling and kept in a germinator at the usual 20-30° C. alternation of temperatures (30° C. for 6 to 8 hours, and 20° C. for the remainder of the day).

<sup>4</sup> ASSOCIATION OF OFFICIAL SEED ANALYSTS OF NORTH AMERICA. RULES FOR SEED TESTING. AS recommended at its 10th annual meeting, 1917. Revised 1921. (Mimeographed).

## COTTONSEED OF THREE CLASSES

After much study of the problem, it was clear that in cottonseed there are three distinct classes of samples:

1. Strong samples in which a large percentage of the seeds will germinate well under a wide range of conditions (Pl. 1, A).
2. Samples which contain many dead or weak seeds which always fail to produce healthy seedlings (Pl. 2, A).
3. "Sensitive" samples, in which a large percentage of the seeds are viable and capable of producing seedlings, although there is a tendency for the seeds to succumb under unfavorable conditions of germination (Pl. 1, C).

The first two classes offer no difficulties to the analyst, so it is the third class which interests us here. It is especially important because a large per cent of the samples of some varieties received from Texas during the past two seasons have belonged to this "sensitive" class.

When sensitive samples are germinated by the standard method, the entire test is often covered by a growth of fungus, many of the young sprouts are water-soaked and decayed, and in many of the remaining seeds the hypocotyl decays before it has a chance to break through the seed coat. From the fact that other samples of the same variety, grown on the same tray, show no tendency to mold, and consistently give high germination results, it is clear that the mold and decay do not result from contamination of the substratum or of the germinator.

## RELATION OF MOLDS AND BACTERIA

An examination by Dr. Chas. Dreschler, of this Bureau, of seedlings from both soil and laboratory germination tests of sensitive samples failed to reveal the presence of any organisms definitely connected with recognized diseases of cotton. However, a beginning has been made of a study of the various organisms actually present on the seeds and their possible relation to the decay of the seeds and seedlings.<sup>5</sup> On the surface of the seeds were found many species of bacteria, and several fungi, the most common being *Rhizopus nigricans*. Surface sterilization of the dry seeds with  $\text{Hg Cl}_2$  (1:1,000) for 30 minutes gave completely sterile seeds. However, seeds which had been in the germinator for 24 hours or longer and were then surface sterilized in the same manner, revealed the presence within the seeds of *Rhizopus* sp. and numerous bacteria—all common soil organisms.

## SENSITIVE SAMPLES NOT THE RESULT OF "HEATING" IN STORAGE

It was at first suspected that the tendency of some samples of cottonseed to mold and decay during germination was due to "heating" in storage. Although a study of the moisture content and temperature of stored cottonseed in Texas in the fall of 1922 gave no evidence of the occurrence of "heating" of the seed, sensitive samples were very prevalent in that season. Damage to cottonseed from "heating" does occur, as has been described in a report of the Arkansas Agricultural Experiment Station,<sup>6</sup> but "heating" of the stored seed is not the cause of the "sensitive" condition of much Texas cottonseed.

<sup>5</sup> The writers are indebted to N. R. Smith, of this Bureau, for help with materials and methods, and for the identification of organisms.

<sup>6</sup> KNAPP, B.—THE EFFECTS OF METHODS OF HARVESTING AND STORING ON COTTONSEED. Ark. Agr. Exp. Sta. Bul. 181: 23. 1922.

## TEMPERATURE FOR GERMINATION OF COTTONSEED

As a result of a study of the effect of temperature on the laboratory germination of cottonseed, it was concluded that the excessive molding of sensitive samples is not dependent on the temperature of germination, and that the usual 20°–30° C. (68°–86° F.) alternation (30° C. for 6 to 8 hours and 20° C. for the remainder of the day) is probably the best temperature of cottonseed germination. Cottonseed can be germinated satisfactorily at 20° C. (although slowly) at 25° C. (77° F.) and at a 15°–35° C. (59°–95° F.) alternation (35° C. for 6 hours and 15° C. for 18 hours).

A comparison of the usual 20°–30° C. alternation with a similar 18°–32° C. alternation and with the reverse alternation (18° C. for 6 to 8 hours and 32° C. for the remainder of the day) indicated that the usual 20°–30° C. alternation is the most satisfactory both for the rate and for the completeness of germination.

## DEMONSTRATION OF "LIVE SEEDS" IN SENSITIVE SAMPLE

As has been indicated above, the "sensitive" samples have been designated as such because a large percentage of the seeds can by special methods be demonstrated to be viable although germination by the standard method gives a low and variable result. It was found that the "live seed" in these sensitive samples could be determined by any one of the following special methods: By testing the seeds in soil with controlled moisture content and controlled temperatures; often by soil tests in the greenhouse; by tests in sphagnum moss; to some extent by "presoaking" the seeds; by surface sterilizing the seeds; and by simply wetting the fuzz of the seeds before putting them to germinate. It would seem that any method which will get water into the seeds quickly and start germination promptly will prevent the development of mold and the decay of the sprouts.

With the soil method, it is difficult to obtain a soil which will uniformly supply the seeds with sufficient moisture. Greenhouse testing in soil is satisfactory if the temperature can be maintained between 60° and 80° F. Surface sterilization of the seeds, either by a dilute solution of mercuric chlorid or by dusting with dry copper carbonate powder (as suggested by Prof. A. H. Leidigh of the Texas Agricultural Experiment Station) will usually control the mold and decay during the germination of these sensitive samples. However, the writers have always hesitated to use a sterilizing agent in making tests of the germinating power of commercial seeds. Although for the prompt germination of cotton the seeds must absorb a large amount of water quickly, an excess of water interferes with germination, probably by limiting the supply of oxygen. For this reason, presoaking the seeds is uncertain, as often a part of the sample is rendered dormant by the process. The simplest and most successful treatment of sensitive samples was found to be a thorough wetting of the fuzz of the seeds before they were put to germinate. This has been designated the "prewetting method." This insures prompt germination of the seeds and in most cases entirely prevents the spread of mold and the decay of the young sprouts.

A few exceptional samples have been encountered which behave in a peculiar manner when tested by the prewetting method. In these samples, many of the seeds remain in a dormant condition in the germinator, as they do when soaked too long in water. In these cases, the total germination is greater by the standard method. Wherever it has been possible to trace the history of such samples, it has been found that the development of the seed has been checked either by weather conditions or by insect attack. Fresh seeds, which still contain a large amount of moisture, behave in the same way.

The difference in the germination results obtained by the two methods is indicated best by a few examples. A typical sensitive sample germinated 71 per cent and 54 per cent in two tests by the standard method; however, the sample contained a large per cent of live seed as indicated by germinations of 95 and 94 per cent, respectively, by the prewetting method. On the other hand, a typical strong sample germinated 95 per cent by the standard method and 98 per cent after prewetting. Prewetting the seed not only prevents the molding of sensitive samples, but greatly hastens the germination of all samples (Pl. 1, A and B). Evidence of the prevalence of sensitive samples, and of the effect of prewetting is shown by the results of one hundred and fourteen tests taken at random from the reports of the Texas Branch of the Seed Testing Laboratory; by the standard method the one hundred and fourteen tests averaged 66.9 per cent germination, by the prewetting method 87.5 per cent. Of these 114 tests, 93 were of typically sensitive samples. Of the remaining 21 tests, 4 were of samples from Louisiana, 7 were separate tests of a single strong sample, 6 were of other strong samples, and 4 indicated samples with a large proportion of dead seeds.

The effect of prewetting on the germination of the three types of samples is shown in Plates 1 and 2, Plate 1, A and B, show No. 483—a strong sample. The germination by the standard method (A) is as good as by the prewetting method (B), although slower. Plate 1, C and D, shows No. 446—a sensitive sample. The excessive mold when germinated by the standard method (C) is typical of this type of sample. The vigorous clean germination after prewetting (D) indicates the high percentage of live seeds. In contrast to the above, the poor germination by both methods (A, standard method) (B, prewetting method) of a typical weak sample, No. 375851, is shown in Plate 2, A and B.

### HOW TO USE THE PREWETTING METHOD

In the use of the prewetting method, the seeds are put in a glass jar with enough water to cover. This is shaken vigorously until the seeds appear uniformly dark colored, indicating that all the air has been displaced from the fuzz and that the surfaces of the seeds are thoroughly wet. Then they are drained and the film of excess water blotted off (on account of the danger of causing dormancy because of too much water). The seeds are then put between folds of wet paper towels and germinated at the usual 20°–30° C. (68°–86° F.) alternation. It has not been possible to standardize the amount of shaking, as some samples are more difficult to wet than others.

During the course of these investigations, the substratum for germinating cottonseed was changed from cloths to an absorbent quality of paper toweling. The germination has been found practically identical with either material, but the convenience and economy of the paper makes it very desirable.

### FIELD BEHAVIOR OF SENSITIVE SAMPLES

Although we found that the sensitive samples contained a large proportion of live seed, which could be germinated by various simple methods, we were uncertain how to interpret to the planter of the seed the results of the germination tests. It was necessary to know the behavior of sensitive samples in comparison with strong samples under different field conditions. Accordingly, we selected, for field study, six samples which had been tested in the laboratory under a wide range of conditions. Arrangements were made through the co-operation of the Office of Crop Acclimatization and Adaptation Investigations of this Bureau and of the Texas Agricultural Experiment Station, to have these samples planted at Lawton, Okla.; Greenville, San Antonio and College Station,

Tex., as well as at the Arlington Experiment Farm near Washington, D. C. Five hundred seeds of each sample were spaced about two inches apart by hand at each planting. From three to six plantings, about a week apart, were made at each station. It was hoped in this way to study the effect of a variety of environmental conditions on the field germination of sensitive samples. The laboratory behavior of the six samples used in the field tests is described in Table I.

TABLE I.—Description of the six samples of cottonseed used in the field tests<sup>a</sup>

No.	Variety	Source	General behavior in laboratory tests	Germination just before sending out for the field tests		
				Standard method	"Pre-wetting" method	In soil in germinators
483	Lone Star...	Clarksville, Tex...	Uniformly high germination; no tendency to mold.	<i>Per cent</i> 94	<i>Per cent</i> 97	<i>Per cent</i> 95
499	.....do.....	Manchester, N. C.	Generally high germination; occasionally slight tendency to mold.	93	96	94
G-40	.....do.....	Greenville, Tex...	Somewhat "sensitive".....	84	91	93
446	.....do.....	Mesquite, Tex....	Very "sensitive." Heavy mold when tested by standard method.	64	93	97
C-7	Mebane.....	Lockhart, Tex....	"Sensitive." Considerable mold when tested by standard method.	71	95	97
5114	.....do.....	.....do.....		79	97	99

<sup>a</sup> All seed of 1922 crop except No. 446, which was grown in 1921 and stored in the laboratory for about eighteen months.

When tested in soil in the laboratory or by the prewetting method, these samples were equally good, but when tested by the standard method, two samples were strong and the other four were sensitive in various degrees.

Their relative behavior under field conditions is summed up in Table II.

TABLE II.—Average field germination of the six selected samples of cottonseed. The germination for each sample is averaged from several successive plantings at each of five stations

Station	Number of plantings averaged	Average germination for each sample					
		Sample No. 483	Sample No. 499	Sample No. G-40	Sample No. 5114	Sample No. C-7	Sample No. 446
Arlington, Va.....	5	<i>Per cent</i> 72.6 a (64-80.8)	<i>Per cent</i> 62.2 (45.8-78)	<i>Per cent</i> 61.8 (52.8-72.6)	<i>Per cent</i> 55.9 (51-60.6)	<i>Per cent</i> 50.9 (41-64.8)	<i>Per cent</i> 40.2 (32.6-53)
Lawton, Okla.....	5	70.9 (58.8-75.8)	58.8 (51.2-64.4)	58.4 (47.4-66.8)	57.5 (44.2-68.8)	61.9 (56.6-73.2)	31.4 (27-40.8)
Greenville, Tex.....	6	70.6 (61.4-85)	63.7 (37.8-79.2)	61.1 (48-66.2)	52.7 (44.2-59.4)	48.2 (31-58.4)	37.1 (30-43)
San Antonio, Tex.....	3	83.3 (78.4-86.6)	74.9 (72-79.4)	74.2 (65.6-80.2)	66.3 (60.2-69.8)	68.9 (60.2-73.8)	45.5 (34.4-58.4)
College Station, Tex.....	3	70.3 (68.8-71.4)	77.1 (69.6-87.2)	64.5 (59.2-71.8)	61.6 (58-65.6)	67.2 (56-79.2)	36 (25-49.6)
Average of all plantings.	22	72.8 a(58.8-86.6)	65.6 (37.8-87.2)	62.9 (47.4-80.2)	57.6 (44.2-69.8)	57.4 (31-79.2)	37.6 (25-58.4)

<sup>a</sup> The numbers in brackets represent the extremes of germination of the individual plantings that enter into the average.

The results of the individual plantings at each station showed considerable variation, depending on the conditions at the time of planting. This is especially noticeable in the germination of the sensitive samples. The germination results for each of the successive plantings at Greenville, Tex., are given in Table III as illustrations of the variations obtained.

TABLE III.—*Summary of the average field germination of cottonseed for successive plantings at Greenville, Tex., 1923*

Sample No.	Apr. 9	Apr. 16	Apr. 23	May 2	May 7	May 15	Average
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
483.....	61.4	85.0	73.6	79.8	62.4	61.4	70.6
499.....	37.8	75.6	51.8	79.2	78.4	59.6	63.7
G-40.....	57.0	64.4	66.2	65.4	65.6	48.0	61.1
5114.....	44.2	52.8	54.4	55.6	59.4	49.8	52.7
C-7.....	31.0	44.2	52.6	56.0	58.4	47.2	48.2
446.....	31.2	38.6	38.8	43.0	41.4	30.0	37.1

In the results of this field study of cottonseed germination, a few points stand out clearly. The strong samples, especially No. 483, gave consistently better stands than the sensitive samples. Sample No. 446 was consistently the lowest germinator in the field, as it had been in the laboratory. It is clear that, on the whole, these sensitive samples are not so poor as would be indicated by the result of the standard test. They are capable of producing a good stand of plants when field conditions are favorable. On the other hand, they are not so valuable as the strong samples, as would be indicated by the result of the test by the pre-wetting method, for they were decidedly less able to withstand extremes of temperature or of moisture. In this connection, it should be emphasized that even the best samples of cottonseed are very sensitive to conditions of germination in the field and may fail to give a stand under unfavorable weather or cultural conditions.

## REPORTS ON THE LABORATORY GERMINATION

As has been pointed out, a test by the prewetting method will determine the number of live seed and distinguish sensitive samples from samples of truly low viability. Although the standard method gives very variable results with these samples, it distinguishes them from the strong samples, and, in connection with the determination of the live seed, gives a very rough idea of the quality of the sample. With these points in mind, the following provisional procedure for reporting cottonseed germination has been adopted by our laboratory.

Each sample is tested by both the standard method and the prewetting method. In addition to the report of the standard test, the result of the prewetting test is reported as "live seed." Each report is accompanied by a brief explanation of the reason for the two tests and their significance. This same procedure is being followed by the Texas State Department of Agriculture.

## MOISTURE CONTENT OF STORED COTTON SEED

The moisture content of the stored seed has been found to be an important factor in the germination of sensitive samples. As the moisture content falls below about ten per cent there is a gradual increase in the tendency of the sample to mold and decay during germination. Moreover, if the seeds dry out until the moisture content is between five and six per cent, there is a marked development of "hard seeds," which fail to take up water at all. A sample from Sacaton, Ariz., was so dry when received that it contained



many hard seeds. Also samples which are kept in a heated laboratory rapidly lose moisture and give increasing trouble in germination. The moisture content of this dry cottonseed can be raised by sprinkling the seed with a small amount of water (about two gallons of water to one hundred pounds of seed), which should be evenly distributed by a thorough mixing of the mass of seed. Then the pile of seeds should be covered with canvas and left for a week to ten days. This moistened seed should be used promptly and not kept for any length of time. Under this treatment the hard seeds are rendered capable of taking up water from the soil, and the rapidity and uniformity of germination are greatly increased. (See Pl. 2, C.) An unusually dry sample, when treated as described and planted in a rather dry soil in the field, germinated 65 per cent in 7 days, while the untreated portion germinated only 29 per cent in the same time.

Prewetting or presoaking cottonseed before planting in the field has not increased the field germination where the soil moisture was good. However, it greatly hastens germination where the soil moisture is not sufficient for the prompt sprouting of untreated seed but where there is enough moisture to maintain the seedlings after germination. From the experience of H. C. McNamara in Texas and in New Mexico, it is suggested as a promising method to be used with caution under special conditions.

### SOME SUGGESTIONS FROM THE PRELIMINARY STUDY

In a discussion of the results of this preliminary study two viewpoints are to be kept in mind—that of the seed analyst and that of the grower or planter.

Any seed analyst who may have occasion to make germination tests of cottonseed, at least of that grown in the Southwest, should recognize the existence of sensitive samples. It would seem to be only fair to the seed grower and the planter of the seed to distinguish these sensitive samples from undoubtedly strong samples and from samples containing a considerable percentage of dead seeds. This distinction may be made by making two germination tests as outlined above. However, the report of the test should indicate that the sensitive samples are not so valuable as the strong samples. Analysts should endeavor to test samples promptly when received, or, if this is not possible, the samples should be protected from the loss of moisture, since excessive drying increases the tendency of sensitive samples to mold during germination, and the results of the test will not represent the true condition of the original lot of seed.

Both the grower and the planter of cottonseed should recognize that all cottonseed, even of the highest quality, is more sensitive to conditions of germination, both in the laboratory and in the field, than the seed of most field crops. It is important that great care should be exercised both in harvesting and in storing to insure sound seed. However, no information is now available to indicate how the occurrence of sensitive seed may be avoided. This phase of the subject is being worked on at present. When the seed analyst's report indicates that planting seed is of the sensitive class, it should be planted only when the temperature and moisture of the soil are very favorable for the germination of cottonseed.

Cottonseed that is very dry will not germinate as promptly or vigorously as that containing a higher moisture content (approximately 12 per cent) at the time it is planted. On the other hand, it is undoubtedly true that during storage the dryer seed will keep its vitality longer. If, at planting time, the seed is excessively dry, it is suggested that the promptness of germination can be increased by artificially raising the moisture content of the seed as described above. In most sections the increased moisture of the atmosphere which prevails in the spring will be sufficient to bring the seeds to a suitable condition for planting.

## PLATE 1

Comparative germination of strong (A and B) and sensitive (C and D) samples by the standard and "prewetting methods."

A.—No. 483, standard method.

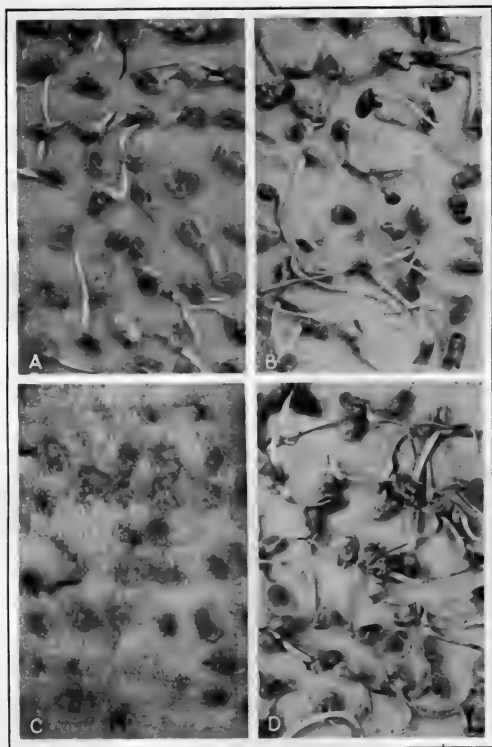
B.—No. 483, prewetting method.

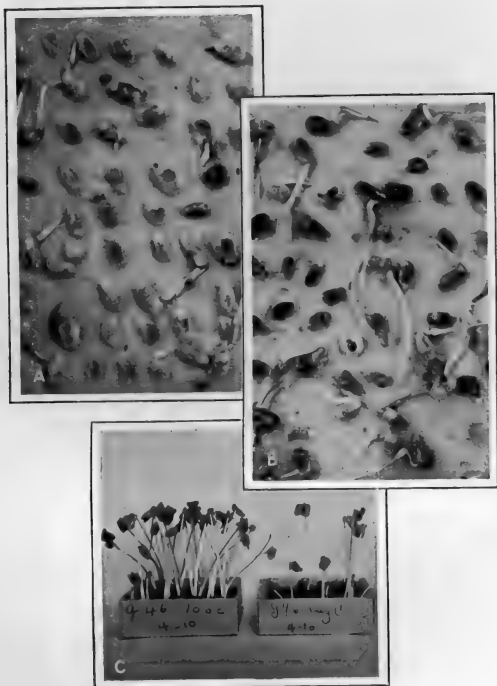
Note that germination is good by either method, but is somewhat more advanced by the prewetting method.

C.—No. 446, standard method.

D.—No. 446, prewetting method.

Note the high percentage of "live seed" indicated by the prewetting method and the low germination and excessive growth of mold when this "sensitive" sample is germinated by the standard method.





## PLATE 2

Germination of a weak sample (A and B) and relation of moisture content of seed to germination (C).

A.—No. 375851, standard method.

B.—No. 375851, "prewetting" method.

The seeds which do not germinate by the standard method are dead, and germination is not improved by the prewetting method.

C.—No. G-46, germinated in soil in the germinators.

The seed planted in the box on the right was very dry when received from Sacaton, Ariz. (Moisture content, 5.7 per cent.) The seed planted in the box on the left was from the same lot, but the moisture content had been raised to 14.7 per cent by the method described in the text (p. 291).

## THE SALT CONTENT OF COTTON FIBER<sup>1</sup>

By THOMAS H. KEARNEY, *Physiologist in Charge, Alkali and Drought Resistant Plant Investigations*, and C. S. SCOFIELD, *Agriculturist in Charge, Western Irrigation Agriculture, Bureau of Plant Industry, United States Department of Agriculture*

It has been reported that the fiber of Pima cotton, a long staple variety of the Egyptian type grown under irrigation in Arizona, is difficult to spin when the humidity of the air is high. The suggestion has been made that this might be due to a high salt content of the fiber, attributable to the fact that irrigated land in the Southwest often has a higher salt content than most of the soils on which cotton is grown in the Southern States.

Determination of the actual salt content of Pima fiber therefore seemed advisable. For this purpose samples of this cotton were collected in 1923 at the United States Field Station at Sacaton, Ariz., together with samples of other types of cotton grown at the same station. Samples (for which the writers are indebted to D. M. Simpson of the Office of Crop Acclimatization and Adaptation, Bureau of Plant Industry, United States Department of Agriculture), were also obtained from James Island, S. C., to permit a comparison of eastern-grown with Arizona-grown cotton.<sup>2</sup> In order to avoid contamination with dust, the fiber was extracted from bolls which were mature, as shown by the fact that they were beginning to crack at the tip, but in which there had been no opportunity for dust to accumulate.

The fiber which enters commerce is picked from fully open bolls and usually accumulates some dust before it is gathered. If commercial Pima cotton showed an abnormally high salt content, this might be due to the deposition of dust on the fiber in the open bolls, rather than to absorption of salt from the soil solution through the roots of the plant. To test this possibility a sample of dusty Pima fiber was collected from fully open bolls at the edge of a field near a road.

Determinations of salt content of the fiber were made on samples collected from the following eight plantings of cotton, the fiber having been extracted from ripe but unopen bolls in all cases except A:

- A.—Pima Egyptian, open bolls, fiber dusty, Peoria, Ariz.
- B.—Pima Egyptian, plants stunted by alkali, Sacaton, Ariz.
- C.—Pima Egyptian, well-grown plants in good soil, Sacaton, Ariz.
- D.—Sea Island cotton, growing adjacent to C.
- E.—Meade Upland cotton, growing adjacent to D.
- F.—Lone Star Upland cotton growing near C, D, and E.
- G.—Sea Island cotton from James Island, S. C.
- H.—Meade Upland cotton from James Island, S. C.

Each sample of fiber was divided into two subsamples, one being used for determination of the water soluble salt content and the quantity of ash not soluble in water, the other for determination of the total ash.

---

<sup>1</sup> Received for publication Mar. 20, 1924.

<sup>2</sup> The soil of the low-lying islands off the coast of South Carolina probably has a somewhat higher content of sodium chlorid than most cotton soils of the Southern States, but the locality where these samples were collected is typical of the area which produces the choice "crop lot" Sea Island cotton.

The water extracts were prepared by soaking 10 gm. of the dry fiber in 100 cc. of distilled water. After 24 hours' immersion, 5 cc. of the extract was withdrawn and was made up to a volume of 50 cc. by the addition of distilled water. The electrical resistance of the diluted extract was then determined and from the resistance, corrected to a temperature of 60° F., the salt content was computed as a percentage of the original 10 gm. of dry fiber.<sup>3</sup>

The fiber used in preparing the extracts was then washed thoroughly with distilled water to remove all water soluble salts, dried and ashed and the weight of the ash was computed as a percentage of the dry weight of the fiber.

From each of the subsamples which had not been leached, 10 gm. of dry fiber were ashed and the ash content of each subsample was computed as a percentage of the dry fiber.<sup>4</sup>

The results of the several determinations are given in Table I.

TABLE I.—*Water-soluble salts and ash in samples of cotton fiber, all values being given as percentages of the dry weight of the fiber*

Samples of fiber	Water-soluble salts, computed from electrical resistance	Ash residues of water-washed samples	Sums of values in columns 1 and 2	Ash residues of un-washed samples	Volatile salts (values in column 3 minus values in column 4)
<b>ARIZONA SAMPLES</b>					
A.—Pima from open bolls.....	1.57	0.38	1.95	0.99	0.96
B.—Pima from unopen bolls, alkali soil.....	1.73	.28	2.01	1.44	.57
C.—Pima from unopen bolls, good soil.....	1.58	.22	1.80	1.23	.57
D.—Sea Island from unopen bolls, good soil.....	1.30	.31	1.61	1.05	.56
E.—Meade from unopen bolls, good soil.....	1.39	.36	1.75	1.33	.42
F.—Lone Star from unopen bolls, good soil.....	1.53	.22	1.75	1.03	.72
<b>SOUTH CAROLINA SAMPLES</b>					
G.—Sea Island from unopen bolls.....	1.44	.20	1.64	1.10	.54
H.—Meade from unopen bolls.....	1.29	.21	1.50	-----	-----
Average of all samples.....	1.48	.27	1.75	1.17	.62

It is evident from the data given in Table I that, as grown on similar soil in Arizona, the salt content of the fiber of Pima cotton (Sample C) is not appreciably higher than that of Sea Island and Upland cottons (Samples D, E, F).<sup>5</sup> Nor do Sea Island and Upland cottons (Samples D and E) grown on irrigated land in Arizona contain appreciably more salt than cotton of the same varieties (Samples G and H) grown in South Carolina. There is somewhat more salt in fiber of Pima cotton from a very salty field (Sample B) than in fiber of the same variety from good soil (Sample C). It is further shown that Pima fiber which has been exposed to dust (Sample A) did not have a higher salt content than fiber of the same variety from unopen bolls (Sample C).

The data in Table I show that on the average 85 per cent of the total salts and ash in cotton fiber is water-soluble material, while 67 per cent is nonvolatile material.

<sup>3</sup> The table used in computing the salt content from the electrical resistance, corrected to 60° F., is given by DAVIS, R. O. E., and BRYAN, H., THE ELECTRICAL BRIDGE FOR THE DETERMINATION OF SOLUBLE SALTS IN SOILS. U. S. Dept. Agr. Bur. Soils Bul. 61, p. 27-29. 1910.

<sup>4</sup> The writers are indebted for the ash determinations to J. W. McLane of the Office of Biophysical Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.

<sup>5</sup> On the other hand, Harris and his associates have found the leaf tissue fluids to contain significantly higher quantities of total electrolytes and of chlorids in Pima Egyptian than in Upland cottons.

HARRIS, J. A., and others. THE TISSUE FLUIDS OF EGYPTIAN AND UPLAND COTTONS AND THEIR F<sub>1</sub> HYBRID. Jour. Agr. Research 27: 267-328, illus.

— LAWRENCE J. V., and LAWRENCE, Z. W. THE CHLORID CONTENT OF THE LEAF TISSUE FLUIDS OF EGYPTIAN AND UPLAND COTTONS. Jour. Agr. Research. [Not yet published.]

The further question arises whether the slightly higher salt content of Pima fiber grown in saline soil (Sample B) as compared with that of fiber from non-saline soil (Sample C) might result in an appreciably greater hygroscopicity, which might affect the spinning properties of the fiber. Also, it may be asked whether the hygroscopicity is reduced by removal of the water-soluble salts.

To answer these questions another portion of each sample (B and C) was divided into three subsamples of approximately equal weight. These were dried at a temperature of 228° F. until no further loss of moisture occurred and the dry weight was determined. The samples were then exposed to an atmosphere having a relative humidity of 65 to 70, at a temperature of 70° F., until they showed no further increase of weight. Their moisture content was then computed by subtraction of the dry weight from the final moist weight. They were next soaked 24 hours in distilled water, washed thoroughly with distilled water to remove the soluble material and dried to constant weight. The washed samples were then exposed to a moist atmosphere under precisely the same conditions as before, and when they ceased to gain weight, their moisture content was again computed. The quantities of water absorber hygroscopically, before and after washing, stated as percentages of the dry weights of the samples, are given in Table II.<sup>6</sup>

TABLE II.—*Dry and moist weights and percentages of water absorbed hygroscopically, before and after removal of the water-soluble salts, of samples of Pima cotton grown on saline and nonsaline land at Sacaton, Ariz.*

Sample and subsample	Before washing			After washing		
	Dry weight	Weight after exposure to a moist atmosphere	Percentage of water absorbed	Dry weight	Weight after exposure to a moist atmosphere	Percentage of water absorbed
B.—From saline soil:	<i>Grams</i>	<i>Grams</i>		<i>Grams</i>	<i>Grams</i>	
1.....	2.86	3.10	8.4	2.76	2.98	8.0
2.....	2.86	3.10	8.4	2.76	2.98	8.0
3.....	2.86	3.10	8.4	2.77	2.99	7.9
Average.....	2.86	3.10	8.4	2.76	2.98	8.0
C.—From nonsaline soil:						
1.....	2.87	3.10	8.0	2.80	3.01	7.5
2.....	2.86	3.10	8.4	2.76	2.99	8.3
3.....	2.85	3.10	8.8	2.76	2.99	8.3
Average.....	2.86	3.10	8.4	2.77	3.00	8.0

Comparison of the results, as given in Table II, shows that the capacity of Pima cotton fiber for hygroscopic absorption of water is not materially affected by removal of the readily water-soluble salts, the unwashed samples having absorbed, on the average, only 0.4 per cent atmospheric moisture more than the washed samples. It would seem, therefore, that the hygroscopicity of the salts present in the unwashed fiber is practically negligible. It is equally clear that the fiber produced by plants growing in saline soil (Sample B) had no higher capacity for hygroscopic absorption of moisture than the fiber produced by plants growing in nonsaline soil (Sample C).<sup>7</sup>

It is concluded that the difficulties said to be encountered in spinning Pima cotton under certain conditions of the atmosphere are not attributable to the salt content of the fiber.

<sup>6</sup> The writers are indebted to Horace H. Willis, of the Office of Crop Acclimatization and Adaptation Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, for the determinations of dry and moist weight.

<sup>7</sup> The loss of weight of the samples as a result of washing averages 0.10 gm. or 3.5 per cent of the dry weight before washing (Table II). The content of electrolytes in Samples B and C, as given in Table I, column 1, averages 1.65 per cent of their dry weight. It appears, therefore, that electrolytes constituted approximately one-half of the water soluble material in these samples of cotton fiber.



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# JOURNAL OF AGRICULTURAL RESEARCH

## CONTENTS

	Page
Studies on Curly-Top Disease of the Sugar Beef - - - - - EUBANKS CARNSER and C. F. STAHL	297
Pecan Scab with Special Reference to Sources of the Early Spring Infections J. B. DEMAREE	321
Anthelmintic Efficiency of Carbon Tetrachlorid in the Treatment of Foxes KARL B. HANSON and H. L. VAN VOLKENBERG	331
The Chemical Composition of Edible Viscera from Meat-Producing Animals WILMER C. POWICK and RALPH HOAGLAND	339
Fumigation of Bean Weevils, <i>Bruchus obtectus</i> Say and <i>B. quadrimaculatus</i> Fab. A. O. LARSON	347
Leather Rot of Strawberries - - - - - DEAN H. ROSE	357
The Cambium Curculio, <i>Conotrachelus anaglypticus</i> Say - - - - - FRED E. BROOKS and R. T. COTTON	377
Relation of the Molecular Proportions in the Nutrient Solution to the Growth of Wheat - - - - - M. C. SEWELL	387
Studies on Nonarsenical Stomach-Poison Insecticides - - - - - WILLIAM MOORE and F. L. CAMPBELL	395

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No. 4

## STUDIES ON CURLY-TOP DISEASE OF THE SUGAR BEET<sup>1</sup>

By EUBANKS CARSNER, *Pathologist, Office of Sugar-Plant Investigations, Bureau of Plant Industry*, and C. F. STAHL, *Assistant Entomologist, Office of Truck-Crop Insects, Bureau of Entomology, United States Department of Agriculture*

### INTRODUCTION

Curly-top<sup>2</sup> of sugar beets presumably was widespread before it was recognized as a distinct disease. It appears to have been confused at first with other diseases or other types of injury. The extensive losses in California, in 1899, referred to as the "California beet disease," "Western blight," etc., led to its being recognized as a distinct and serious disease, and the following year a similar condition was determined as occurring in all of the Western States where sugar beets were grown.

Linhart (9)<sup>3</sup> summarized the reports of several specialists with reference especially to California conditions. This report recorded the conditions of the areas infected but did not establish the cause or exact character of the injury.

Townsend (19) described and figured curly-top.

Ball (1) in the Annual Report for 1905 of the Utah Station announced the discovery of a causal relation between the feeding of the beet leafhopper and "curly-leaf" or "blight."

Smith (14) reported the severe outbreak of 1905 in California, stating that the injury was apparently physiological in character and possibly of the same general type of disease as tobacco mosaic.

Ball (2) emphasized, by further observation, the fact that "curly-leaf" is the result of leafhopper attack. He suggested that the disease itself might be in the nature of gall formation.

Wilhelmi (21) reported alkali injury as a possible cause of curly-top.

Townsend (20) described curly-top from both the pathological and geographical standpoints; while reaching the conclusion that the disease was a distinct systemic disease, he concluded that as yet no satisfactory explanation of the cause had been found.

Ball (3) adopted the name "curly-leaf" for the disease, reviewed the earlier work, and reported in considerable detail the relation of the sugar beet leafhopper and other leafhoppers to this and other injuries to the host plants. He emphasized especially that the curly-leaf condition, though produced by the leafhopper, is not due to mechanical injury by the insect nor to loss of sap. He pointed out that, in the case of injury produced in plants by related species of leafhoppers, the injury was apparently local in character; while in the case of the curly-leaf, the abnormal condition apparently spread from leaf to leaf, infecting the whole plant even though the leafhopper might have disappeared in the meantime. He also reported that the agency that caused curly-leaf was capable of remaining in the beet over winter, again producing the typical curly-leaf symptoms in the first leaves in the spring. He referred to the experimental production of "curly-leaf" in cages arranged jointly by himself and Prof. E. G.

<sup>1</sup> Received for publication Jan. 23, 1924.

<sup>2</sup> The name "curly-top" has been used in earlier publications of this Department. The question of the advisability of employing "curly-leaf" instead of "curly-top" as the common name of this disease has been referred to the American Phytopathological Society.

<sup>3</sup> Reference is made by number (*italic*) to "Literature cited," p. 318-319.

Titus, by H. B. Shaw and by the Office of Sugar-Beet Investigations, Bureau of Plant Industry, United States Department of Agriculture.

Shaw (13) confirmed Ball's findings regarding the occurrence of curly-top in sugar beets as a result of infestation by beet leafhoppers. He caged aphids, red spiders, thrips and beet leafhoppers on sugar beets and found that curly-top developed only in the beets on which the beet leafhoppers were caged. Shaw also reported that, even though no symptoms of the disease were visible when the beets were harvested the preceding fall, curly-top disease developed during the second season in beets planted for seed production, and that the symptoms of the disease were to be found primarily upon the young or growing leaves.

Bunzel (7) reported a high oxidase content in the tissues of diseased beets, recalling the high oxidase content reported in earlier experiments on tobacco mosaic.

Smith and Bonquet (16) reported that exposure of beets for five minutes to infestation by leafhoppers was sufficient to cause the symptoms of curly-top to develop, although two weeks or more might be necessary before the symptom would be obvious. They reported a bacillus as associated with the disease.

Bonquet and Hartung (5) reported beet leafhoppers not necessarily able to cause the disease while, if such leafhoppers were first fed on diseased beets and then allowed to feed on healthy beets curly-top would then develop.

Smith and Bonquet (15) reported the finding of bacteria in the diseased beets and confirmed the report of Bonquet and Hartung of the apparent inability of the leafhopper to transmit curly-top unless it had previously fed upon diseased plants.

Ball (4) urged the use of the name "curly-leaf" to clearly distinguish between the disease associated with leafhopper attack and any disease having symptoms in any way similar. He reviewed the status of curly-top or "curly-leaf," especially emphasizing the geographical relation of the breeding areas of the beet leafhopper to the areas in which severe outbreaks had occurred. He also emphasized the periodicity of migrations from breeding areas and suggested the probability that epidemics of curly-leaf could be predicted through the recognition of the periodicity of the flights of the insect. Commenting on the finding by Bonquet and Hartung that the insects were apparently unable to carry the disease unless they were fed upon diseased plants, he suggested the probability that some wild plant was acting as carrier of the disease.

Bonquet and Stahl (6) reported the occurrence of the curly-top infection in native vegetation.

Stahl and Carsner (17) reported that recently emerged beet leafhoppers, lifted from the plant before feeding were not carriers of curly-top, but became carriers of the disease when fed on diseased beets.

Severin (10) stated that the insects caught on wild vegetation produced the disease in beets in some cases while in other cases they did not. He listed wild plants from which the leafhoppers were reared and from which these insects transmitted curly-top to sugar beets.

Carsner (8) reported fourteen species of plants representing eight families as susceptible to curly-top.

Severin (11) demonstrated that after a nonviruliferous insect has fed on a diseased plant an incubation period for at least a few hours must elapse before this insect can transmit the virus.

Stahl and Carsner (18) reported that when an insect is once infected the infection is apparently permanent.

The appearance of sugar beets affected with curly-top in the different stages of the disease might well be more adequately described, but for the purpose of this paper brief mention of some of the conspicuous symptoms will suffice. When a plant is infected the mature leaves remain normal in appearance, the vein swellings and curling of the leaves appearing on only the new growth. There is no conspicuous color change in the early stages of the disease (Pl. 1). In the later stages the older leaves which were mature at the time of infection,

turn yellow and die, while the younger central leaves which are dwarfed and usually show conspicuous vein swellings on the under sides remain green (Pl. 2, D). The vein swellings vary with different individual plants from cases where practically every veinlet is irregularly swollen (Pl. 2, B), to cases which show only scattered, inconspicuous, rounded swellings (Pl. 2, A). The disease is characterized by a necrosis of the phloem throughout the vascular system (Pl. 3, B), and in the advanced stages of the disease this necrosis shows in the roots as dark concentric rings (Pl. 4). The exudation of a clear, viscous liquid is often conspicuous on the oldest leaves which show the vein swellings (Pl. 2, C).<sup>4</sup>

### DISTRIBUTION OF CURLY-TOP AND THE BEET LEAFHOPPER

The geographical distribution of the curly-top disease is limited to the arid or semiarid regions of the western part of North America in which the insect *Eutettix tenella* Baker is found and to the districts adjacent to these regions. In general it may be said, therefore, that the disease occurs wherever this leafhopper is found. It has been authoritatively reported from the following States: Arizona, California, Colorado, Idaho, Kansas, Nebraska, New Mexico, Oregon, Texas, Utah, and Washington.

The disease has been reported from practically every region in California in which beets are grown. The distribution of the beet leafhopper has been studied more extensively than has the distribution of the disease, and because of its significance from the standpoint of where the disease occurs or would occur if beets were planted the localities from which the insect has been reported are listed here.

### LOCALITIES FROM WHICH THE BEET LEAFHOPPER HAS BEEN REPORTED

#### CALIFORNIA

- |                         |                       |                              |
|-------------------------|-----------------------|------------------------------|
| 1. Agatha.              | 36. Dos Palos.        | 70. Oxnard.                  |
| 2. Allensworth.         | 37. Earlimart.        | 71. Pacheco Pass (entrance). |
| 3. Alpaugh.             | 38. El Centro.        | 72. Paso Robles.             |
| 4. Altamont Pass.       | 39. Famoso.           | 73. Pleasanton.              |
| 5. Alvarado.            | 40. Gonzales.         | 74. Prado.                   |
| 6. Angiola.             | 41. Goshen Junction.  | 75. Ravendale.               |
| 7. Antelope Valley.     | 42. Hamilton City.    | 76. Red Bluff.               |
| 8. Arlington.           | 43. Heber.            | 77. Riverside.               |
| 9. Bakersfield.         | 44. Helm.             | 78. Rosamond.                |
| 10. Barstow.            | 45. Hemet.            | 79. San Ardo.                |
| 11. Betteravia.         | 46. Holtville.        | 80. San Bernardino.          |
| 12. Bitterwater.        | 47. Huntington Beach. | 81. San Fernando Valley.     |
| 13. Blanco.             | 48. Huron.            | 82. San Jacinto.             |
| 14. Brawley.            | 49. Imperial.         | 83. San Jose.                |
| 15. Buena Vista.        | 50. King City.        | 84. San Lorenzo Creek.       |
| 16. Buttonwillow.       | 51. Lancaster.        | 85. Santa Ana.               |
| 17. Calexico.           | 52. Le Grand.         | 86. Santa Margarita.         |
| 18. Calipatria.         | 53. Los Alamitos.     | 87. Sargent.                 |
| 19. Castaic.            | 54. Los Banos.        | 88. Saugus.                  |
| 20. Castroville.        | 55. Lost Hills.       | 89. Shafter.                 |
| 21. Centerville.        | 56. McKittrick.       | 90. Soledad.                 |
| 22. Chino.              | 57. Manteca.          | 91. Spence.                  |
| 23. Cholame.            | 58. Marysville.       | 92. Spreckels.               |
| 24. Chowchilla.         | 59. Mendota.          | 93. Stockton.                |
| 25. Chualar.            | 60. Merced.           | 94. Tejon Pass.              |
| 26. Claremont.          | 61. Milpitas.         | 95. Tracy.                   |
| 27. Coalinga.           | 62. Modesto.          | 96. Victorville.             |
| 28. Compton.            | 63. Mojave River.     | 97. Visalia.                 |
| 29. Connor.             | 64. Moro Cojo.        | 98. Volta.                   |
| 30. Corcoran.           | 65. Mountain View.    | 99. Wasco.                   |
| 31. Coyote Wells.       | 66. Neenach.          | 100. Watts.                  |
| 32. Crows Landing.      | 67. Niland.           | 101. Westmoreland.           |
| 33. Davis.              | 68. Norwalk.          | 102. Williams.               |
| 34. Dixieland.          | 69. Oro Loma.         | 103. Willowbrook.            |
| 35. Dominguez Junction. |                       |                              |

<sup>4</sup> The progress of the work has been greatly aided by the use of the laboratory and field facilities of the Citrus Experiment Station of the University of California. The writers are indebted to the director and the staff for many courtesies and accommodations.

## OREGON

- |               |               |           |
|---------------|---------------|-----------|
| 1. Echo.      | 3. Pendleton. | 4. Union. |
| 2. La Grande. |               |           |

## WASHINGTON

- |                  |               |               |
|------------------|---------------|---------------|
| 1. Ellensburg.   | 4. Pasco.     | 6. Toppenish. |
| 2. Granger.      | 5. Sunnyside. | 7. Wenatchee. |
| 3. North Yakima. |               |               |

## IDAHO

- |               |                 |                 |
|---------------|-----------------|-----------------|
| 1. Blackfoot. | 6. Idaho Falls. | 10. Payette.    |
| 2. Buhl.      | 7. Jerome.      | 11. Rupert.     |
| 3. Burley.    | 8. Nampa.       | 12. Shelley.    |
| 4. Gooding.   | 9. Oakley.      | 13. Twin Falls. |
| 5. Hansen.    |                 |                 |

## NEVADA

1. Fallon.

## UTAH

- |                      |                |                   |
|----------------------|----------------|-------------------|
| 1. Austin.           | 8. Lynndyl.    | 15. Richfield.    |
| 2. Box Elder County. | 9. Mills.      | 16. St. George.   |
| 3. Delta.            | 10. Monroe.    | 17. St. Joseph.   |
| 4. Elsinore.         | 11. Moroni.    | 18. Salina.       |
| 5. Fillmore.         | 12. Nephi.     | 19. Sigurd.       |
| 6. Garland.          | 13. Ogden.     | 20. Spanish Fork. |
| 7. Lehi.             | 14. Panguitch. | 21. Thompsons.    |

## ARIZONA

- |              |              |
|--------------|--------------|
| 1. Glendale. | 2. Prescott. |
|--------------|--------------|

## COLORADO

- |                  |                    |                |
|------------------|--------------------|----------------|
| 1. Fort Collins. | 3. Grand Junction. | 4. Rocky Ford. |
| 2. Lamar.        |                    |                |

## NEBRASKA

1. Grand Island.

## TEXAS

- |              |             |
|--------------|-------------|
| 1. Amarillo. | 2. El Paso. |
|--------------|-------------|

## NEW MEXICO

1. Las Cruces.

The localities listed include those recorded by Ball (1), (4) and Severin (11) and unpublished reports by W. J. Hartung, G. T. Scott, and the present writers.

## ECONOMIC IMPORTANCE OF CURLY-TOP

The studies on curly-top by the writers have been conducted in the main in California, and so the consideration of the economic importance here given is based principally on California conditions. The fact should be borne in mind, however, that the disease frequently causes serious losses in the beet-growing regions of other Western States. In the regions where beets have been grown in California the losses may in general be said to have varied (within limits) directly with the distance from the coast. In cooler coastal regions or so-called fog belts the damage is usually slight or relatively unimportant. As one goes inland into the progressively warmer and more arid districts the damage caused by the disease increases so that nearly every year the resulting losses are serious and in the so-called "bad years" the losses vary from very serious to total. The losses have been so serious in some of the interior districts that vast available areas otherwise suitable for beet growing have been completely abandoned after several years of unsuccessful efforts to grow beets on an extensive commercial scale. Curly-top was, of course, not the only difficulty encountered in these hot, dry districts, but, in the opinion of the writers, it was the limiting factor.

A more definite conception of the extent of the losses which the disease has caused may be formed from Table I. These figures were kindly furnished by sugar companies operating in California. They apply to districts which the writers have had under observation. The losses, therefore, are known to be due principally to curly-top. The yield per acre for these districts in years when curly-top has not been serious has ranged from 10 to 15 tons. The losses caused by the disease may be represented graphically by showing the net yield of beets, in tons per acre, in the four localities for each of the years considered (fig. 1).

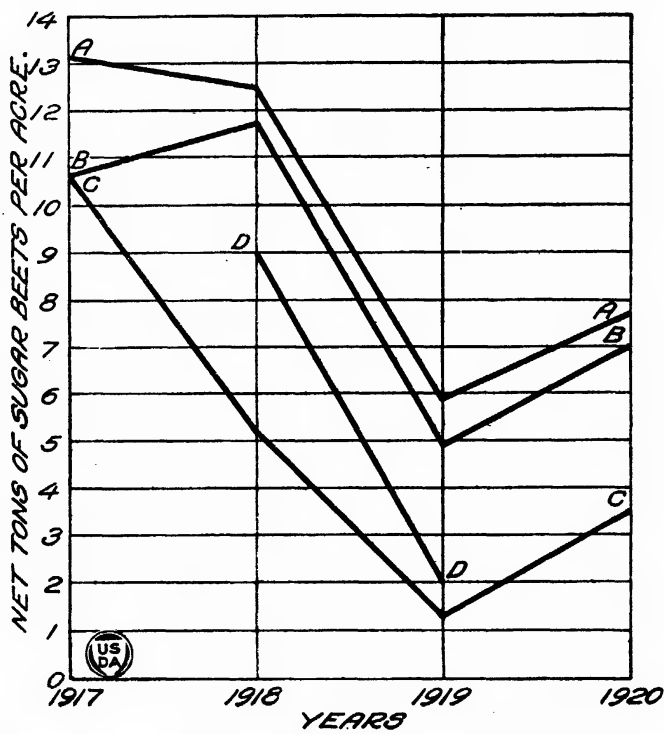


FIG. 1.—Graph showing the relative losses caused by curly-top disease

TABLE I.—Losses caused by curly-top in four localities in California

Locality and year	Crop area (acres)		Gross yield (tons)		Tare		Net yield per acre (tons)	Yield of sugar	
	Plant-ed	Har-vested	Har-vested	Per acre	Per cent	Tons per acre		Per cent	Calcu-lated tons per acre
District A:									
1917	1,307	1,287	17,774	13.8	5.0	0.7	13.1	19.3	2.5
1918	915	915	13,216	14.4	13.2	1.9	12.5	17.7	2.2
1919	1,288	1,288	8,349	6.5	8.2	.5	5.9	19.8	1.2
1920	933	933	7,882	8.4	8.2	.7	7.8	19.7	1.5
District B:									
1917	320	320	3,576	11.2	5	.6	10.6	19.4	2.1
1918	400	400	5,231	13.1	10.5	1.4	11.7	16.8	2
1919	675	675	3,748	5.6	8.13	.5	5.1	19	1
1920	490	470	3,601	7.7	8.4	.4	7.2	18.7	1.4
District C:									
1917	3,293	3,291	37,149	11.3	5.8	.6	10.6	19.2	2
1918	3,145	2,930	17,970	6.1	15.26	.9	5.2	16.8	.9
1919	3,425	2,550	3,664	1.4	9.18	.1	1.3	19.3	.3
1920	3,042	2,939	11,220	3.8	7.6	.3	3.5	19.8	.7
District D:									
1918	1,136	1,136	10,535.18	9.3	2.9	.3	9	17	1.5
1919	786	786	1,711.13	2.1	7.6	.2	2	17.1	.3



The question might be raised as to the possibility of other factors, such as variation in rainfall, having entered in to cause the great differences in yield. In answer to this question it may be said that irrigation is practiced in all the districts and that the amount of rainfall is therefore probably of no great significance. The rainfall is given, however, for a representative locality in the region of each of the districts A, B, and C to show the amount and distribution of the rainfall in the seasons of 1917, 1918, and 1919 (Table II).

TABLE II.—Rainfall in the region of districts A, B, and C, seasons of 1917, 1918, and 1919

Month	Seasonal rainfall			Month	Seasonal rainfall		
	1916-17	1917-18	1918-19		1916-17	1917-18	1918-19
	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>		<i>Inches</i>	<i>Inches</i>	<i>Inches</i>
September.....	1.50	0	5.17	March.....	0.76	2.90	1.94
October.....	.37	0	.32	April.....	.15	.38	.08
November.....	.55	.71	2.39	May.....	.06	.09	0
December.....	2.40	.31	1.74	Total.....	8.92	7.31	15.66
January.....	.97	.47	.96				
February.....	2.16	2.45	3.06				

The growing of sugar beets as an annual crop is by far the more important phase of the industry in this country at the present time. The preceding discussion of the damage caused by curly-top is only in regard to this phase of the industry. The growing of sugar-beet seed is another phase of the industry in which curly-top is an important factor. Because of the biennial nature of the sugar beet, the plants must grow at least a part of two seasons in order to flower and produce seed. According to the most successful procedure under California conditions the seed is planted in summer or early fall. The young plants, or stecklings, are then transplanted to the seed-beet fields in winter or early spring. In the summer and fall months the stecklings are very apt to become infected if leafhoppers are present. The selection of suitable plants for transplanting is based chiefly on size of roots. Naturally, therefore, many plants which are infected late are on that account about normal in size and are transplanted to the seed fields. The result of this procedure is that a considerable proportion of the transplanted plants fail to produce flower stalks or produce only dwarfed, diseased stalks. An example of the part the disease plays was observed in the seasons of 1918 and 1919. The seed was planted late in the summer of the first year in a region infested with beet leafhoppers. As the stecklings grew up in the late summer and fall it was evident from the number of leafhoppers present and the cases of the disease scattered through the field that a considerable proportion of the plants had been inoculated. The stecklings were transplanted in the following winter and early spring with the result that because of curly-top about 30 per cent of the plants failed to produce seed.

No appreciable injury of seed beets due to infection in the second season of growth has been noted. When healthy stecklings are planted in the winter or early spring they grow to such an extent during the cool months before the leafhoppers appear or become abundant that there is practically no danger of injury from the disease. This observation is based on conditions in California, but it probably holds true also for other districts where seed beets are grown.

In addition to the direct loss due to the damage to the beet crops, great financial loss is involved when sugar factories are built in districts which prove unsuitable. The cost of building and later dismantling and removing the factory to another locality, together with the loss of capital invested in other improvements and equipment, must also be considered. Two instances in California alone are known in which factories were dismantled and removed because of the inability to grow beets on account of curly-top.

## EFFECT OF CURLY-TOP ON GROWTH

A plant that becomes infected while young—when the root is perhaps 1 cm. or less in diameter—will usually die in a relatively short while or before the summer is past, but a plant that is about half grown or larger when infected will usually live a long time, as a rule to the end of the season.

Measurements made during two seasons of the roots of beets affected with curly-top show that increase in size of the root usually continues for a considerable length of time, often more than a month after the plant has become evidently diseased. The data taken during one season are listed in Table III. The plants were grown at Riverside, Calif. Figures are given of the size and number of the leaves of each plant, because the vigor of growth of the root is correlated with the size of the top. Plants which were evidently diseased on May 14 continued to grow, so that by July 26 the roots of most of them had nearly doubled in diameter. The measurements in connection with observations of the affected plants indicate that increase in size of roots continues as long as the older leaves, which were mature at the time of infection and therefore have remained apparently normal, survive. The weight of the top or leaves, as well as that of the root of each plant at the time of digging on July 26, is given. These figures indicate the great retarding effect of the disease on the growth of the tops. It is of interest to note the wide variations in individual plants as to the effect of the disease. The variation appears to be greater than that which occurs in the case of diseases of many plants.

TABLE III.—*Effect of curly-top on the growth of sugar beets*

Plant	Number of leaves on May 14	Maximum leaf and root dimensions							Weight on July 26		Date of observation and degree of disease when first noted
		Leaves on May 14		Root diameters					Top	Root	
		Width	Spread	On May 14	On June 2	Increase in 19 days	On July 26	Increase in 54 days			
No. 3...	10	Cm. 10.2	Cm. 43	Cm. 2.09	Cm. 3.57	Cm. 1.48	Cm. 3.98	Cm. 0.41	Gm. 27	Gm. 78	May 14, two leaves affected. May 14, five youngest leaves affected.
No. 6...	18	13.3	56	3.62	5.53	1.91	6.49	.96	62	207	
No. 8...	16	11.4	52	3.38	5.51	2.13	5.71	.20	49.5	169	May 14, one leaf affected. May 14, three youngest leaves affected.
No. 12...	15	15.9	53	3.10	4.98	1.88	5.85	.87	89.5	272	
No. 19...	17	16.5	64	3.02	4.63	1.61	5.84	1.21	87	185	May 14, seven youngest leaves affected.
No. 20...	15	12.7	62	3.80	5.02	1.22	5.20	.18	43.5	120	May 14, six of younger leaves affected.
No. 22...	18	15	56	3.79	6.35	2.56	7.88	1.53	147	385	May 14, seven of younger leaves affected.
No. 32...	15	13.3	62	2.98	4.50	1.52	5.26	.76	26	125	May 14, three of younger leaves affected.
No. 35...	21	10.8	76	3.90	5.32	1.42	5.54	.22	74.5	114	May 14, nine of younger leaves affected.
No. 36...	19	20.3	70	4.10	5.97	1.87	5.71	-.26	22.5	186	May 14, four of younger leaves affected.
No. 38...	12	10.8	63	2.41	3.08	.67	2.88	-.20	10	20.5	May 14, five of younger leaves affected.
No. 2...	14	10.2	46	2.35	4.70	2.35	7.12	2.42	132	276	June 2, one young leaf affected.
No. 4...	8	6.4	47	1.19	3.02	1.83	4.54	1.52	55	112	June 2, four of younger leaves affected.
No. 5...	15	17.8	51	2.77	5.56	2.79	7.63	2.07	153	417	June 2, eight youngest leaves affected.
No. 10...	10	8.3	58	1.23	3.10	1.87	3.60	.50	22	48.5	June 2, six youngest leaves affected.
No. 11...	19	15.9	61	4.62	7.49	2.87	8.06	.57	90	440	June 2, four youngest leaves affected.
No. 13...	11	14	53	3.25	5.78	2.53	6.94	1.16	175	380	Do.
No. 14...	10	15	66	3.03	5.60	2.57	7.67	2.07	300	440	June 2, five of younger leaves affected.

TABLE III.—Effect of curly-top on the growth of sugar beets—Continued

Plant	Number of leaves on May 14	Maximum leaf and root dimensions							Weight on July 26 (grams)		Date of observation and degree of disease when first noted
		Leaves on May 14		Root diameters							
		Width	Spread	On May 14	On June 2	Increase in 19 days	On July 26	Increase in 54 days	Top	Root	
No. 16...	24	Cm. 12.7	Cm. 66	Cm. 3.99	Cm. 7.28	Cm. 3.29	Cm. 7.61	Cm. 0.33	Gm. 52	Gm. 252	June 2.
No. 23...	12	14.6	60	2.72	5.25	2.53	6.70	1.45	83	207	June 2, two youngest leaves affected.
No. 26...	20	17.8	61	4.69	8.69	4.00	10.82	2.13	185	753.5	June 2.
No. 27...	19	14	69	3.89	6.76	2.87	8.72	1.96	152.5	449	June 2, three of younger leaves affected.
No. 28...	16	20	74	3.61	6.40	2.79	8.20	1.80	169	300	June 2, seven of younger leaves affected.
No. 31...	15	16.5	81	4.15	6.99	2.84	8.13	1.14	19	425	June 2, about half of the leaves affected.
No. 37...	9	8.3	55	1.60	3.36	1.76	4.32	.96	22	64	June 2, one young leaf affected.
No. 9...	16	16.5	69	3.93	7.90	3.97	10.23	2.33	321	802	June 2, healthy.
No. 15...	12	14.6	77	2.69	5.25	2.57	6.71	1.46	68	300	Do.
No. 17...	13	21.6	79	5.83	9.76	3.93	10.95	1.19	301.5	896.5	Do.
No. 24...	13	10.2	53	2.18	4.79	2.61	8.24	3.45	225	438	Do.
No. 30...	13	10.2	48	3.11	5.22	2.11	6.46	1.24	116	306.5	Do.

DRY WEIGHT OF TOPS INCREASED

Moisture determinations which were made at three different times of the tops or leaves of young healthy and diseased beets show that the proportion of dry weight of the tops of young diseased plants exceeds the proportion of dry weight of those of young healthy plants by 2.5 to 3 per cent. This excess of dry weight is attributed to the excessive accumulation of carbohydrates, probably sugars. Due to the breaking down of the phloem, normal translocation is impossible. The clear, viscous exudate (Pl. 2, C) has been repeatedly found by Fehling's test after hydrolysis with an acid to contain a complex sugar, probably saccharose. In only one or two instances were traces of reducing sugars detected. The escape of the exudate by rupturing the tissue is attributed to the high pressure developed by the abnormal accumulation of the sugars.

TABLE IV.—Artificial inoculations of sugar beets, resulting in no disease <sup>a</sup>

Date	Kind of inoculum	Kind and number of plants inoculated	Method of inoculation
Season of 1918:			
March 3....	Fresh excreta of the leaf hopper.	Two young beet seedlings.	Point of steel needle dipped into drops of excreta and then pricked into plants.
August 3...	Ringer's solution extract and aqueous extract of young leaf of curly-top beet.	Four young beet seedlings.	Sprayed each extract on two plants; then caged on each five nonviruliferous leafhoppers.
August 12...	Six adult leafhoppers macerated in 2 cc. of Ringer's solution.	Three young beets.	A drop of inoculum placed on each of two leaves of each plant and pricked in with small steel needles.
Do.....	Curly-top beet leaf macerated in Ringer's solution.	.....do.....	Do.
Do.....	Curly-top beet leaf macerated in distilled water.	.....do.....	Do.
Season of 1919:			
May 24....	Pieces of curly-top beet leaf...	Eight beets (these had five or six true leaves).	Placed tissue on midrib near base of blade, macerating over and pressing into a slit in the midrib.
August 8...	Pieces of petiole of leaf of curly-top beet.	Seven young beets.	Placed tissue on center of blade of one leaf of each and then macerated into wound in the blade with scalpel and needle.

<sup>a</sup> Since this manuscript was prepared curly-top has been artificially communicated by transferring juice from diseased to healthy beets, as described in the following publications: SEVERIN, H. P. CURLY LEAF TRANSMISSION EXPERIMENTS. *Phytopathology* 14: 80-93, illus. 1924; CARSONER, E., and STAHL, C. F. PROGRESS REPORT ON CURLY-TOP OF THE SUGAR BEET. (Abstract) *Phytopathology* 14: 122-123. 1924.

TABLE IV.—*Artificial inoculations of sugar beets, resulting in no disease—Contd.*

Date	Kind of inoculum	Kind and number of plants inoculated	Method of inoculation
Season of 1919— Continued.			
August 8....	Entire viruliferous leafhopper.	Seven young beets.	One insect macerated on and pricked into a leaf of each plant. Insect first stunned by a gentle pressure between the fingers.
August 13....	Two entire curly-top plants macerated in distilled water.	Three young beets.	About 500 cc. of the extract poured on the soil around the roots.
Do.....	Part of the preceding extract.	Several young plants.	Plants dug with as little injury to the roots as possible and roots placed in extract for one hour; older leaves then removed and plants replanted.
August 27....	Distilled-water extract of two entire curly-top beets.	Several beet plants with roots one-fourth inch in diameter.	Roots placed in the extract for one hour; older leaves then removed and plants replanted.
October 2....	Pieces of midrib of badly affected leaf of a curly-top beet.	One young beet..	Tissue placed in a slit in the petiole; wound not covered.
Do.....	Piece of midrib of curly-top beet leaf.	One beet seedling..	Tissue placed in a slit in the hypocotyl; wound covered with soil.
Do.....	A leafhopper nymph from a curly-top beet.	One young beet....	Insect forced into a slit in the petiole of the plant; wound bound with cloth and grafting wax.
Do.....	Adult leafhopper from a curly-top plant.	One beet seedling..	Insect pressed into a slit in the hypocotyl; wound wrapped and covered with grafting wax.
Do.....	Entire viruliferous insect.....	Three beet plants with $\frac{1}{2}$ -inch roots.	One insect in a slit in the crown of each plant just below the ground level.
October 18....	Small piece of petiole of a leaf of a curly-top beet.	Three young beets..	Tissues placed in a slit in the root just below ground level; wound covered with soil.
October 21....	Tops and roots of curly-top beets separately extracted in distilled water.	Eight plants with $\frac{1}{2}$ -inch roots.	Roots allowed to stand in extract over night; leaves then removed and roots replanted.
Season of 1920:			
April 27.....	Extract of entire curly-top plant in distilled water.	Several beets.....	Healthy leaves cut and cut ends placed in vials of the extract; non-viruliferous leafhoppers placed on these for two days and then on healthy beets.
May 2.....	Tap-water extract of two entire young curly-top beets which had been macerated in a mortar.	Three young beet plants	Slowly injected through fine glass nozzles attached to a funnel containing the extract, which was so elevated as to cause a gentle pressure of the liquid.
May 12.....	Tap-water extract of two entire curly-top beets which had been macerated in a mortar.	Two young beet plants.	Cut ends of petioles of healthy leaves placed in vials of the extract; seven nonviruliferous insects were then caged on the blades of these leaves for three days and then transferred to healthy plants.
May 15.....	Expressed juice of 12 young curly-top beets.	Several young beets.	Plants dug and roots allowed to stand in the juice for four hours and then replanted.
Do.....	Expressed juice of curly-top plants and expressed juice diluted with equal parts of tap water.	.....do.....	Roots placed in the two solutions for four hours and then replanted.
Do.....	do.....	.....do.....	Cut ends of petioles of healthy leaves placed in vials of the extract and nonviruliferous insects placed on them for four days and then transferred to healthy beets.
July 5.....	Parts of viruliferous leafhoppers which had been anesthetized with ether to facilitate dissection.	Twenty-one young beets.	Parts of insects (e. g., heads, thorax, and abdomen) were placed in Ringer's solution, then on each plant an insect part was inoculated into the base of the blade of each of the first true leaves by means of a sharp steel needle.
Season of 1921:			
May 3.....	Juice of a curly-top plant extracted by means of a hypodermic syringe.	Seven beet plants. One had only cotyledons; the others had seven leaves.	Juice slowly injected by means of hypodermic syringe immediately after it was extracted from diseased plant. Injected into crowns and cut end of a petiole.

TABLE V.—Inoculation of sugar beets with insects other than *Eutettix tenella*, no disease resulting

Season, number, and date	Species of insect	Number of insects used	Length of time insects were on curly-top beets	Healthy plant to which the insects were transferred	Length of time insects were left on healthy plants
Season of 1915:					
No. 1, Aug. 30	<i>Empoasca viridescens</i> .....	5	4 days.....	Young beet.....	28 days.
No. 2, Aug. 30	<i>Agallia</i> spp.....	2	do.....	do.....	Do.
No. 3, Aug. 30	<i>Thamnotettix geminatus</i> (?).....	1	do.....	do.....	Do.
No. 4, Aug. 30	<i>Cicadula 6-notata</i> .....	2	do.....	do.....	Do.
No. 5, Aug. 30	<i>Liburnia</i> sp.....	1	do.....	do.....	Do.
No. 6, (?)	<i>Empoasca viridescens</i> .....	(?)	Collected from diseased plants.	do.....	22 days.
No. 7, (?)	<i>Agallia</i> spp.....	(?)	Collected from diseased plants in the field.	do.....	Do.
Season of 1917:					
No. 8, Aug. 29	<i>Empoasca viridescens</i> .....	7	47 days.....	(a).....	47 days.
No. 9, Aug. 29	<i>Agallia cinerea</i> .....	7	35 days.....	(a).....	35 days.
No. 10, Sept. 4	<i>Empoasca viridescens</i> .....	6	39 days.....	(a).....	39 days.
No. 11, Sept. 4	<i>Nysius</i> sp.....	6	29 days.....	(a).....	29 days.
No. 12, Sept. 4	White fly ( <i>Aleyrodidae</i> ).....	12	do.....	(a).....	Do.
Season of 1917:					
No. 13, Sept. 14	<i>Aphis</i> sp.....	Large.		(2) (b).....	
No. 14, Sept. 14	do.....	Large.		(3) (c).....	
No. 15, Sept. 4	<i>Lygus pratensis</i> .....	10	29 days.....	(a).....	29 days.
Season of 1919:					
No. 16, May 22	<i>Acinopterus</i> sp.....	3	4 days.....	Young beet.....	15 days.
No. 17, May 22	<i>Chlorotettix</i> sp.....	3	do.....	do.....	Do.
No. 18, May 22	<i>Agallia</i> sp. (large).....	(?)	do.....	do.....	Do.
No. 19, May 22	<i>Agallia cinerea</i> .....	3	13 days.....	do.....	44 days.
No. 20, May 22	<i>Empoasca viridescens</i> .....	5	20 days.....	do.....	37 days.
No. 21, July 1	<i>Thamnotettix montanus</i> .....	4	4 days.....	do.....	27 days.
No. 22, (?)	<i>Aphis</i> sp.....	Large.	3 days.....	(d).....	
No. 23, Apr. 28	<i>Eutettix strobil</i> .....	3	2 days.....	Young beet.....	2 days.
Season of 1920:					
No. 24, Feb. 23	<i>Empoasca viridescens</i> .....	6	Several days.....	Young beets and <i>Stellaria media</i> .	18 days.

<sup>a</sup> The insects were placed in a cage with a small curly-top beet and a small healthy beet for the number of days stated.

<sup>b</sup> A curly-top beet leaf heavily infested with aphids was placed in a cage with healthy and curly-top plants. The insects colonized on both plants.

<sup>c</sup> A curly-top beet leaf heavily infested with aphids was placed in a cage with a healthy plant upon which the insects colonized.

<sup>d</sup> A curly-top leaf with aphids on it was removed to a cage with a healthy beet and another to a cage with two healthy *Stellaria media* plants.

Observational evidence agrees with these negative results from attempts to transmit the disease by insects in so far as aphids and *Empoasca viridescens* are concerned. These two species have been repeatedly observed infesting the plants in cages which contained both curly-top and healthy plants and in no case has there been any evidence that they transmitted the virus of the disease.

The question of transmission by grafting perhaps should be considered in this connection. Smith and Bonquet (15) claim to have transmitted the disease by grafting parts of diseased beets on to healthy plants. Thus far the present writers have been unable to successfully graft parts of a curly-top plant on to a healthy plant, and the attempts to make these grafts have not produced the disease.

### IS THERE AN INCUBATION PERIOD IN THE LEAFHOPPER?

As has previously been stated, *Eutettix tenella* is the only known agent or means by which the virus of curly-top can be transmitted. Also, the fact has been pointed out that the ability to produce the disease is not an inherent characteristic

of the insect. In view of these facts the question as to whether or not an appreciable interval of time must elapse after a nonviruliferous<sup>5</sup> leafhopper has fed on a diseased plant before it is able to effectively transmit the virus may have some significance regarding the nature of the virus. Smith and Boncquet (15) state that a period of at least 24 hours but not more than 48 hours must elapse. Severin (11) worked with batches of 25 to 50 insects instead of single leafhoppers. He found that at high temperatures (about 100° F.) the insects were occasionally able to produce the disease within four to six hours after they had been placed on a diseased plant. For this short period his results showed 8 cases of disease from 60 tests. No positive results were obtained from 36 tests for a period of one to three hours. Periods of 24 to 48 hours after the insects were placed on the diseased plant gave a greater proportion of positive results than shorter ones. The experimental tests which the writers have thus far made in regard to this question are listed in Table VI. In each of these experiments nonviruliferous leafhoppers were placed on a curly-top beet for a given number of hours and then transferred individually to young healthy plants for a certain number of hours and then transferred again, etc. For example, in experiment No. 1 the non-viruliferous insects were caged on a curly-top plant for 24 hours and then caged singly on healthy plants for the same length of time. The first period tested was from 24 to 48 hours after the insects were placed on the diseased plant. After that the insects or those which still survived were transferred to other healthy plants for 24 hours. This second period, therefore, was from 48 to 72 hours after the insects were placed on the diseased plant. The successive transfers of the same insects are indicated in Table VI by attaching the letters *a*, *b*, *c*, etc., to the number of the experiment.

The results show that some of the insects were able to transmit the virus within a period of 21 hours and 45 minutes after they were placed on the diseased beet. In another case the period was 22 hours and 20 minutes, and in a third case it was 23 hours. In some cases the insects all failed to produce the disease within periods of 24 hours or less. A point which may be of some significance is that the results seem to indicate that a greater number of the insects become able to transmit the virus after a longer period than 24 hours than are able to do so in the shorter time. The facts cited seem to indicate that a multiplication of the causal agent takes place within the insect.

#### RELATION OF THE NUMBER OF INSECTS TO CURLY-TOP

Comparative inoculations were made at the same time and on similar plants with different numbers of insects. The object was to determine whether or not the quantity of virus injected was significant in producing the disease or in regard to the period of incubation. Twelve experiments have been performed to test this. The procedure in these tests was so varied that the details can not be readily tabulated. The results, however, may be summarized. They indicate that when 10 insects are used the inoculated plant is more likely to become diseased than when the inoculation is made with 1 or 2 insects. It seems probable that this fact is due to the variation of individual insects in regard to infection rather than to the quantity of virus injected. In the cases where the disease is produced there is no difference in the incubation period or severity of the effect between inoculations with single insects and those with 10 insects.

<sup>5</sup> It has recently been brought to the writers' attention that the term *viruliferous* occurs in medical literature; and as it appears to have the same meaning *viruliferous* (meaning virus-bearing) as used by the writers in previous publications, it is of course desirable to use *viruliferous* for the sake of preventing a duplication of terms.

TABLE VI.—Length of incubation period in the leafhopper before becoming viruliferous

Date started	Experiment No.	Length of time			Number of insects		Remarks
		On curly-top plant	On healthy plants	Total	Tested	Producing the disease	
Season of 1918:		Hours	Hours	Hours			
July 17.....	1a	24	24	48	20	1	Inoculated plants in 5-inch pots.
Do.....	1b	-----	24	72	14	5	Plants in bed in green-house.
							More favorable conditions for growth may have been a factor.
Do.....	2a	24	24	48	20	5	
Do.....	2b	-----	24	72	20	9	One insect which produced the disease in the first period failed to do so in the second period, so that five more than in the first period were then effectively viruliferous.
August 14.....	3a	24	24	48	30	5	
Do.....	3b	-----	24	72	30	6	
September 2.....	4a	-----	24	48	30	10	<i>Stellaria media</i> plants were used instead of beets.
Do.....	4b	-----	24	72	28	22	One insect which caused disease in the first period did not cause it in the second, so there was in the second period an increase of 13 that were effectively viruliferous.
Do.....	4c	-----	24	96	26	15	
November 8.....	5a	5	18	23	26	11	<i>Stellaria media</i> plants were used instead of beets.
Do.....	5b	-----	73	96	24	22	The second period was so much longer than the first that a fair basis of comparison is hardly afforded.
Season of 1920:							
August 23.....	6a	3	19½	22½	5	-----	
Do.....	6b	-----	9	31½	5	-----	
Do.....	6c	-----	15	46½	5	1	
Do.....	6d	-----	72	120	5	1	
Do.....	7a	7	15½	22½	4	1	
Do.....	7b	-----	9½	31½	4	3	
Do.....	7c	-----	15	46½	4	-----	
Do.....	7d	-----	72	120	4	2	One of the four insects did not produce the disease at all.
September 13.....	8a	5½	17	22½	10	-----	
Do.....	8b	-----	7½	30	10	1	Insect No. 8 produced the disease.
Do.....	8c	-----	17½	47½	10	3	Nos. 5, 7, and 10, produced the disease.
Do.....	8d	-----	144	192	10	4	Nos. 6, 7, 9, and 10, produced the disease; four of the insects did not produce the disease at all.
September 14.....	9a	4½	17½	21½	10	2	Nos. 2 and 7 produced the disease.
Do.....	9b	-----	7½	29½	10	-----	
Do.....	9c	-----	72	96	10	3	Nos. 2, 5, and 7 produced the disease; seven of the insects did not produce the disease at all
September 22.....	10a	6	2½	240	10	-----	
Do.....	10b	-----	15	23½	5	-----	
Do.....	10c	-----	8	31½	10	1	No. 10 produced the disease.
Do.....	10d	-----	6	47½	10	2	Nos. 1 and 6 produced the disease.
Do.....	10e	-----	8	55½	10	1	No. 2 produced the disease.
Do.....	10f	-----	16½	72	10	-----	
Do.....	10g	-----	240	312	10	4	No. 1, 4, 6, and 10, produced the disease; five of the insects did not produce the disease at all.

VARIATION OF INDIVIDUAL INSECTS IN REGARD TO INFECTION

In the attempt to determine whether or not there is any uniformity of behavior among individual leafhoppers in regard to producing the disease, a considerable number of insects were caged singly on individual plants, under similar conditions, on beets or other susceptible plants for periods of 24 hours or longer on consecutive days. Tests of this nature were repeatedly made, using 3 to 30 individual insects in a test. The results of two of these tests or series are brought together in Table VII.

The question has been raised as to whether the infectivity of the insect or ability to produce the disease runs in cycles, such as might occur if the virus were an organism with a definite life cycle. The results as listed in Table VII seem to give no evidence of definite cycles of infectivity. The evidence seems to justify the conclusion that, in general, when an insect has produced or failed to produce the disease in a given period it is more likely to give a similar result in the subsequent one or more periods before changing to the other result. In other words, the positive or negative results are more likely to be grouped together in consecutive periods than they are to alternate. This idea is based on the results shown in the table, as well as on the results of other tests. As to what is the significance of this seeming tendency of the results, the evidence thus far justifies only speculation. It is of interest to note the preponderance in the number of cases in which the disease was produced over the number of negative results.

TABLE VII.—Variation of individual insects in regard to causing infection  
[The plus sign (+) indicates the production of the disease, and the minus sign (−) indicates that no disease resulted]

Inoculation period	Insect No.																													Total for each period
Hours	6	7	9	12	13	21	22	53	54	55	56	57	58	59	60	61	63	65	66	67	68	69	71	72	73	86	87	88	89	+
24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	6
384	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	6
24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	6
24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	7
24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	5
24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	6
24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	4
24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0
48	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	1
24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	3
24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	2
24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	1
24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	8
48	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	10
24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	8
48	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	10
24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	5
24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
72	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	11
72	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	8
72	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	4
24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	5
48	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	7
48	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	3
48	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	10
48	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	13
120	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	6
24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	7
24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	7
24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	6
48	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	6
72	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	7
24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	4
72	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	3
168	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	4
24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	8
216	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	7
24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	5
Total for each insect:																														
Plus	8	8	7	8	5	7	4	5	3	4	2	11	12	4	15	5	12	9	1	17	5	18	2	19	8	7	1	10	3	220
Minus	3	3	4	3	2	4	1	2	6	2	4	5	10	1	7	1	10	12	5	6	17	5	3	2	13	6	5	2	3	147

The numbers at the top of the columns were used to designate the individual insects. The figures in the left-hand column show the number of hours the insects were on the plants. The plus sign (+) indicates that the disease was produced as a result of the inoculation in that period. Likewise the minus sign (−) indicates that



curly-top was not produced in a given period. The periods represented between any two of the horizontal lines show the number of insects used on any one day or period. Where the columns are very short the fact is to be inferred that the insect concerned soon escaped or died. A blank in a column indicates that no note was obtained for that period, owing to the fact that the tag label could not be found.

RELATION OF LIGHT TO INFECTION

A limited amount of experimental work has been done in the attempt to determine whether or not light is necessary for infection to occur. Young beet plants were kept in darkness for a period and then inoculated in comparison with a series of plants exposed to daylight. In each experiment some of the plants were inoculated on the cotyledons and others on the true leaves. The details of the procedure and the results are listed in Table VIII.

TABLE VIII.—Relation of light to infection of beet plants tested in 1920

Experiment and date	Length of time			Point of inoculation	Number of plants inoculated	Diseased
	In the dark before inoculation	Insects on plants	In the dark			
	Hours	Hours	Hours			
No. 1, Mar. 24-----	48	72	120	Cotyledons-----	5	<sup>a</sup> 2
	48	72	120	First true leaves-----	5	<sup>b</sup> 1
No. 2, Apr. 1-----	26	22	48	Cotyledons-----	3	3
	26	22	48	First true leaves-----	3	1
No. 3, Apr. 24-----	34	21	55	Cotyledons-----	8	3
	34	21	55	First true leaves-----	7	1
	(c)	21	-----	Cotyledons-----	8	2
	(c)	21	-----	First true leaves-----	7	4
No. 4, Nov. 25-----	19	26	65	Cotyledons-----	6	6
	19	26	65	First true leaves-----	6	3
	(c)	20	-----	Cotyledons-----	4	4
	(c)	20	-----	First true leaves-----	4	4
No. 5, Dec. 4-----	22	20	42	Cotyledons-----	4	2
	22	20	42	First true leaves-----	4	0
	(c)	20	-----	Cotyledons-----	4	1
	(c)	20	-----	First true leaves-----	4	2

<sup>a</sup> Only four lived.                      <sup>b</sup> Only two lived.                      <sup>c</sup> Not placed in the dark.

In each test plants of the same age were used throughout, and they were grown under conditions as nearly similar as possible except in regard to light. If any significance may be attached to the results of these few tests, they indicate that, with young plants which have been kept in darkness for about 24 hours prior to inoculation, infection can be more readily effected on the cotyledons than on the leaves. Plants exposed to usual daylight, on the other hand, seem about as easily infected on the true leaves as on the cotyledons. If these facts be true, it would seem logical to presume that the presence of food substance as stored products in the cotyledons or from the process of photosynthesis in all green parts facilitates infection. It seems worthy of note that in test No. 1, where the plants were kept in darkness for five days, only two of the five leaf-hoppers caged on the true leaves survived, while all five of those on the cotyledons lived. Probably those on the true leaves were starved.

EFFECT OF HEAT ON THE VIRUS

The question as to whether or not the virus of curly-top is destroyed by relatively high temperatures was studied by placing nonviruliferous leafhoppers on parts of curly-top beets which had been immersed in water at the desired temperature. The root pieces were cut so that in no case was the thickness greater than 1 cm. A period of 10 minutes was used in each experiment. After the leaves or root pieces had been immersed in the heated water, nonviruliferous insects were caged on these beet parts for 24 hours and then removed to healthy plants. The results of the experiments are shown in Table IX. It should be noted that in the cases where the insects failed to secure the virus from heated leaves the leaves were so decomposed by heat as to be unfit for food. The results of the experiments seem to indicate that the virus is not destroyed by temperatures lower than those at which the beet tissue is killed. Many bacterial parasites of plants are killed by temperatures lower than those which the curly-top virus will endure.

TABLE IX.—*Effect of the application of heat on the virus of curly-top*

Season, experiment, and date	Tem- perature of water used (°C.)	Parts of diseased plant heated	Results
Season of 1919:			
Number 1 { April 29.....	45 to 46	Leaves.....	Plants diseased.
{ April 29.....	54 to 55	do.....	Plants healthy.
Number 2 { May 8.....	45	Leaf.....	Plants diseased.
{ May 8.....	50	do.....	Plants healthy.
Number 3, June 6.....	49 to 50	Leaves.....	Plants diseased.
Number 4, August 7.....	49.5 to 50	do.....	Do.
	51 to 52	do.....	Do.
Season of 1920:			
Number 5, April 14.....	51 to 52	do.....	Do.
Season of 1921:			
Number 6 { October 14.....	51 to 52	Leaves and root pieces.....	Do.
{ October 20.....	52 to 53	do.....	Do.
{ October 20.....	60 to 61	Leaves.....	Plants healthy.
{ October 20.....	60 to 61	Root pieces.....	Plants diseased.

EFFECT OF DESICCATION ON THE VIRUS

Four tests in which dried tissue of curly-top beets was remoistened with water and then placed in cages as food for nonviruliferous leafhoppers have given only negative results. The insects were unable to produce curly-top when removed to healthy plants. The survival of the insects in three of the tests for periods of approximately 24 hours at room temperature indicates that they must have fed to some extent on the old diseased tissue. Further work is needed to justify the conclusion that the virus is destroyed by desiccation.

DISTRIBUTION OF VIRUS IN AFFECTED PLANTS

Those leaves which are mature at the time of infection remain normal or free from symptoms of the disease until they begin to yellow with age. The question has therefore been suggested as to whether or not these apparently normal leaves contained the virus or disease-causing factor. Tests have been made by the use of nonviruliferous leafhoppers. These insects were caged on the leaves to be tested and then transferred to healthy beet plants to determine if they had become effectively viruliferous. Eight trials of this nature have been made. In a few instances the insects failed to secure the virus from the old, normal-appearing

leaves. In most of the trials, however, the insects secured the virus from the normal-appearing leaves as well as from the younger, evidently diseased leaves. The peculiar fact should be noted, however, that the insects which were placed on the leaves showing the symptoms were able to transmit the virus sooner than those insects which were placed on the older, normal-appearing leaves.

The fact that the virus is present in the roots of diseased plants has been repeatedly determined by the use of nonviruliferous insects.

### IS A BACTERIAL ORGANISM ASSOCIATED WITH CURLY-TOP?

The discovery of a bacterial organism associated with beets affected by curly-top was announced by Smith and Bonquet (15) and earlier workers. The work of the present writers on this point may be divided into two phases. In the first place, attempts have been made to grow beet plants under aseptic conditions by the use of steamed soil and disinfected seed. The plants were covered with bell jars to protect them from contamination by air-borne organisms. After curly-top had been produced in some of the plants, pieces of affected leaves of these were transferred, without disinfection, to tubes or plates of a culture medium. Bacteria grew from the leaf pieces in some instances, but the important phase of the results is that in several of the cases the medium into which the curly-top tissue was introduced remained sterile or showed only fungus contamination.

The other phase of the study of the question has been the plating of pieces of tissue of curly-top plants. Pieces of the interior of roots and of petioles have been cut out with aseptic precautions and then plated without disinfection. Pieces of diseased leaves have been plated after disinfection with mercuric chlorid for 5 to 15 minutes. In the majority of the cases both the untreated and the disinfected tissue remained sterile. In some instances bacteria grew from the tissue pieces, but several different types were represented. None of these were tested by inoculation.

The results of these two lines of work indicate at least that no easily culturable specific bacterial organism is constantly associated with curly-top. That bacteria may get into curly-top tissues through the open wounds caused by the dying back of lateral roots and the ruptures on the leaves made by the liquid exudate seems probable.

### THE INCUBATION PERIOD OF THE DISEASE

The length of time that elapses from the inoculation of the plant until the first symptoms appear is considered as the incubation period of the disease. Temperature affects the length of the period, as is noted elsewhere in this paper. The incubation period is shortest when, with high temperatures, conditions are still favorable for growth of the plant. With beet plants in cheesecloth cages out-of-doors in the summer at Riverside, Calif., as short an incubation period as four days has been occasionally noted. With *Stellaria media* an incubation period of four days has several times been observed. In one case with this plant symptoms were visible within 48 hours after inoculation. In general the incubation period in sugar beets varies from 7 to 14 days. With low temperatures or conditions otherwise unfavorable for growth the period may be prolonged, sometimes for a month or more.

The discussion of the incubation period of the disease as given here should not be confused with the discussion by Severin (11). He speaks of the time elapsing between inoculations and when "noninfective" insects were able to secure the causative agent from the plant as the incubation period of the causative agent.

## RELATION OF TEMPERATURE TO CURLY-TOP

The writers' ideas as to the relation of temperature to the incubation and development of curly-top are based principally on observations made under natural conditions at different seasons. Facilities for accurately controlling temperatures where plants were grown in California have not been available, but the evidence obtained indicates that soil temperature as well as that of the air affects the duration of the incubation period in beets. For instance, in an experiment where all the inoculated plants were exposed to the same range of air temperatures but were exposed to two different soil temperatures, symptoms of the disease appeared first at the higher temperature. With the plants where the maximum soil temperature ranged from 25° to 35° C. symptoms of curly-top appeared on the eighth day, while in the case of the plants where the maximum temperature varied from 15° to 20.5° C. symptoms first appeared on the eleventh day. Results of two series of tests conducted with controlled soil temperatures in a greenhouse at Madison, Wis.,<sup>6</sup> support the view as to the influence of soil temperatures. Plants inoculated in California and sent by mail to Madison and there grown at a soil temperature of 33° C. developed the symptoms sooner than did those at 15° C. In the case of *Stellaria media* the results of one test indicate that soil temperature is less important than in the case of the beet. Where the air temperature was the same for both sets of plants the symptoms appeared at the same time in both the high and low temperature soil. If this seeming difference between beets and chickweed holds true it may possibly be explained on the basis of the different structure of the two plants. In the case of the beet the virus has to pass through the crown of the plant, which is usually at or below the soil level, in order to reach the new leaves. The main shoots of a *Stellaria media* plant throw out lateral branches, so that the curly-top virus may reach new leaves without necessarily passing through the root or crown. It would, therefore, probably be influenced in its rate of spread or development chiefly by the air temperature.

Low temperatures retard the progress of the disease as they lengthen the incubation period. This fact probably explains why curly-top is generally less injurious, even when it occurs in the cooler coastal districts, than it is in the warmer regions.

## AGE OF PLANTS IN RELATION TO SUSCEPTIBILITY

The question of the relation of the age of a plant to its susceptibility to curly-top infection is of great interest from the standpoint of cultural practice as to time of planting. Field observations indicate that if beets have attained a considerable size before the leafhoppers appear they are much more likely to make a harvestable crop than they will if very small when the leafhoppers come in. This fact seems due, in part at least, to the beets becoming less easily infected as they grow older. A limited amount of experimental work on the question, which is detailed in Table X, supports this idea. In the experiments where plants of different ages were inoculated on the same date both lots were growing under similar conditions. The age of the plants used is indicated in the table by giving the number of leaves developed.

<sup>6</sup> The details of the tests at Madison were handled by Hurley Fellows.

TABLE X.—Age of sugar beet plants in relation to susceptibility

Season, experiment, and date	Age of plants	Insects per plant	Number of plants	
			Inoculated	Diseased
Season of 1920:				
No. 1, January 2....	6 to 8 leaf stage.....	1	35	22
No. 2, March 13....	{Cotyledon stage.....	1	6	2
	{6 to 7 leaf stage.....	1	6	0
No. 3, March 17....	{Cotyledons and first pair of leaves.....	1	6	4
	{7 to 8 leaf stage.....	1	6	1
No. 4, March 31....	{Cotyledons and first pair of leaves.....	1	15	8
	{6 to 8 leaf stage.....	1	15	1
No. 5, April 2....	{Cotyledons and first pair of leaves.....	1	15	12
	{6 to 8 leaf stage.....	1	15	3
No. 6, July 14....	8-months old.....	2	20	0
Season of 1921:				
No. 7, July 2....	{4 months old.....	2	10	1
	{2 months old.....	2	11	2
No. 8, September 20....	8 to 9 leaf stage.....	2	6	1
No. 9, October 14....	10 to 16 leaf stage.....	2	14	1

## OTHER PLANTS SUSCEPTIBLE TO CURLY-TOP

A list of plants found susceptible to curly-top has been previously given (8). Since that time no extensive effort has been made to determine what other plants may be affected by the disease. The annual (redmaids) *Calandrinia menziesii* T. and G., may now, however, be added to the list of those plants proved to be susceptible (Pl. 5, A and B.) This species is of special interest in this connection because it is one of the early winter annuals and probably serves along with (alfileria) *Erodium cicutarium* in harboring the curly-top virus over winter.

Among cultivated plants the tomato, *Lycopersicon esculentum* Mill, may now be included among the susceptible species. The effects of the disease on the tomato are rather of the mosaic type, a clearing of the veins of the younger leaves which gives these a slightly mottled appearance and a curling and distortion of the youngest leaves. Affected plants gradually wilt, turn yellow, and die within a few weeks after infection. The disease has not been found in commercial plantings, so far as is known to the writers. This is probably explained by the fact that the tomato seems to be unfavorable as a food plant for the leaf hopper. The variety known as Stone was used in the inoculation experiments.

The case of goose-foot, *Chenopodium murale*, is of special interest in considering the susceptibility of other plants. In the list previously published it was given as nonsusceptible. Before the time of that publication, nine experiments in regard to the susceptibility of *C. murale* had been performed. Viruliferous leaf-hoppers were caged on young plants in the attempt to produce the disease, or nymphs reared from viruliferous females on *C. murale* were tested on beets. The results of all these tests indicated that the species was not susceptible to the disease. The fact may be of some significance that all these early experiments were made at Spreckels, Calif., where the climate is usually cool. During the year 1919 and the two subsequent seasons, further tests were made at Riverside, Calif. In some instances the results were in part or entirely positive. In other words, *Eutettix tenella* nymphs reared on *C. murale* from viruliferous females produced curly-top in some or all of the plants to which they were transferred. Out of 43 experiments, however, only 13 gave positive results. The positive results show that under some conditions the plant harbors the virus of curly-top. In no case have clearly defined symptoms, such as swelling of the leaf veins or veinlet clearing, been noted, but in some of the plants a dwarfing of the later growth which may have been due to the disease has been observed. The fact that positive results were obtained in so small a proportion of the later tests and in none of

those done earlier shows that in its relation to this disease the species is peculiar. The climate of Riverside is in general warmer than that of Spreckels, particularly in summer, but opposed to the idea that climatic differences may explain the difference in results in the two localities is the fact that some of the positive results were secured during the winter. It seems probable that the species is resistant to curly-top.

An interesting observation made in connection with two of the experiments seems probably due to this seeming resistance. In two of the tests where nymphs from *Chenopodium murale*, the progeny of viruliferous leafhoppers, were caged on healthy young beets several of the beets soon developed faint clearing of the veins of some of the youngest leaves. The first symptom of curly-top is a clearing of the veins of the younger leaves. In these two tests, however, the vein clearing gradually disappeared in most of the plants and normal growth was continued. Only one or two plants developed typical cases of curly-top. One theory, which seems a plausible explanation, is that by passage through the *C. murale* the curly-top virus was so weakened or modified as to be rendered unable, in most cases, to effectively establish itself in the sugar beet. The point has not as yet been determined as to whether by means of nonviruliferous leafhoppers the virus may be obtained from those beet plants which show the faint vein clearing and subsequently recover.

As to variation in susceptibility to infection, the fact has been previously mentioned (8) that *Stellaria media* seems much more readily infected than the beet or buckwheat. There are some indications that *Erodium cicutarium* and *E. moschatum* and possibly *Calandrinia menziesii* are more readily infected than the beet. These three species, as well as spinach and tomato, die quickly as a result of the disease and in that sense are much more susceptible to the disease than several other species, e. g., *Malva parviflora*, cheese weed, and the sugar beet.

### OVERWINTERING OF THE VIRUS

Few if any of those leafhoppers which leave the beet fields in the fall ever return. The observations by which this fact was established were in the main made in California. An observation made in Utah in the early summer of 1922 supports the belief that the same situation exists in that region. Near Elsinore, Utah, two sugar beet fields were inspected. In one of these fields a few cases of curly-top were found by diligent search, but certainly less than one-half of 1 per cent of the plants were affected. In the other field 1,200 plants were counted without finding any case of the disease. In both of these fields beet leafhoppers were present, and they had been there long enough to allow a considerable number of nymphs, their progeny, to emerge. It was estimated that there were 50 to 75 nymphs on each plant. Although the leafhoppers must have been on the beets for at least a month, there was practically no curly-top. Evidently most of the insects which flew into these fields in spring were nonviruliferous.

The question therefore arises as to where the new brood of insects which enters the fields in spring secures the curly-top virus. The suggestion has been previously made (8) that from susceptible annuals of the natural breeding areas some of these spring-brood leafhoppers may secure the virus. That this is not the only way in which the virus probably passes the winter should be made clear. As mentioned elsewhere in this paper, a small proportion of the leafhoppers usually remain in the cultivated area in the fall after the main body of the insects has departed. These "hold-over" insects, or "stragglers," being viruliferous, infect some of the early planted beets. Beets thus infected then serve as a source of virus for nonviruliferous insects that may come in later.

A third possible way in which the virus may be carried over winter is in old diseased plants growing in or near beet fields. In almost every old beet field such plants can be noted as making renewed growth in the spring. Frequently such plants are affected with curly-top. They would therefore serve as sources of the virus for any nonviruliferous insects that happened to feed on them.

## CONTROL MEASURES

### LEAFHOPPER CONTROL

The control of the beet leafhopper is naturally the first idea suggested for the prevention of curly-top damage, because this insect is the only known agency by which the disease is spread. The only feasible time of attack on the insects is in the early spring, when they first enter the beet fields and before they have deposited many eggs. They must then be killed or caught. The writers have tried the use of a hopperdozer. Large numbers of the insects were caught, but such a considerable proportion escaped that little or no benefit resulted. Liquid and dust sprays as insecticides and repellants have been tried to a limited extent by the writers. Kerosene emulsion in 6, 12, and 18 per cent strengths were tried. No significant decrease in the amount of curly-top resulted, even though some of the insects were killed. Nicotine sulphate in three different strengths gave only similar results. As repellants the following were tested: Bordeaux mixture, oil of citronella, naphthalene diluted with equal parts of lime, and mixtures of sulphur and tobacco dust with lime. In none of these tests was the benefit worthy of consideration. As has been mentioned before, the use of dusts, particularly mixtures containing nicotine sulphate, has been tried by others (12). The results thus far reported are not encouraging from the standpoint of commercial practice.

Control of the leafhopper by means of its natural enemies has been considered. There are three species of egg parasites and at least four parasites of the nymphs and adults. These parasites no doubt hold the leafhoppers in check to a large extent, but they can not be relied upon to prevent the production of curly-top in disastrous amounts.

### TIME OF PLANTING

The control of *Eutettix tenella* by artificial means or by natural enemies seems at best a remote possibility, but by planting the beets early the damage due to this leafhopper can be avoided to a large extent. Emphasis should be placed in this connection on the fact that by early planting is not meant planting as early as possible in the fall, e. g., in October, but rather in the latter part of November and December, or as soon thereafter as practicable, and, for most of the affected regions, not later than February, if possible. Striking evidence in regard to the time-of-planting question was afforded by field observations in California in the season of 1918 at Corcoran and Bakersfield in the San Joaquin Valley and at Lancaster in the Mojave Desert. At Lancaster and Corcoran small plats of beets planted very early in the winter (October and November) were much more seriously diseased than were adjacent plats planted a month or two later. At Bakersfield, where the curly-top injury has usually been great, practically all of the planting of the season of 1917-18 was done in November, December, and January, and a good crop was produced, the beets being well advanced in size before the leafhoppers became abundant. At Corcoran, on the other hand, in the near vicinity of Lake Tulare, where the slow drying out of the soil retarded operations so that planting was delayed, as late as the latter part of March in some cases, great injury from curly-top resulted. In the vicinity

of Riverside and San Bernardino, in southern California, good crops have been produced by early planting (November and December), while the crops from late planting (March) in the same season were practically complete failures.

In regard to the time-of-planting question, from the standpoint of minimizing the injury from curly-top, two points must be given especial consideration. The plantings must not be made early enough in the fall to expose the young plants before the movement of the leafhoppers from the cultivated fields or late enough in the spring so that the beets will not have made a vigorous growth before the leafhoppers move into the cultivated areas. Mention should be made of the fact that usually some of the leafhoppers remain in the cultivated areas through the winter and that these stragglers may cause a considerable amount of damage. In general, however, the damage from those that remain in the cultivated areas, as compared with the damage when the main body of the insects is in cultivated districts, is relatively slight. With varying seasons the optimum time of planting will no doubt vary, but in general the safest plan is to plant as soon as possible after the middle of November and at least before the end of February. This recommendation is based primarily on conditions in California.

#### RESISTANT STRAINS

The fact that in almost every severely affected beet field a few individual plants will stand out conspicuously less affected than the rest has been noted by previous workers. The writers have repeatedly made this same observation. A project has been started by selecting the seemingly resistant plants from commercial fields, believing that by selection and possibly breeding a resistant strain satisfactory for commercial purposes may be developed.

Difficulties have been encountered in keeping over winter the beet roots which had to be removed from the field before harvest time. Siloing the beets in the ground, as is done in colder climates, has been found impracticable. Different methods of cold storage have been tried and a way of packing the roots for this purpose has been tested which seems, from the experience of one year, fairly satisfactory. Direct transplanting of the roots when harvested has been tried to a limited extent and found satisfactory.

Seed was produced from a few of the selected beets in the summer of 1921. Plantings of these strains together with control plats of commercial seed were tested for resistance in the autumn of that year. This test was made at Riverside, Calif., where leafhoppers were naturally abundant. In addition to the natural exposure, each plant of the selected strains was inoculated by caging on it a viruliferous leafhopper. The results of the test gave support to the belief that a strain of beets resistant to curly-top may be developed. Seeming differences in degree of resistance were noted between several of the selected strains and the commercial beets, and in one case this difference was marked (Pl. 5, C). Tests of these strains were made in the same manner but on a larger scale in the summer of 1922. The situation was complicated in this season by the fact that a large proportion of some of the apparently resistant strains developed seed stalks. On this account no satisfactory conclusions could be drawn as to resistance. Work is now in progress to determine the factors causing the premature seeding so that these factors may be eliminated.

More selections were made from commercial fields in California and Utah and also from the progeny test plats in the summer of 1922. The selection of more individuals from commercial fields and from the progeny plats will be continued and the progeny of these selections tested by inoculation for resistance to curly-top. The practice being followed in the growing of seed from the selected strains is to plant the mother beets singly or in small groups in private gardens, so that they will be isolated from each other.



## SUMMARY

Curly-top disease of beets is characterized by a dwarfing of the whole plant, curling of the leaves, and irregular swelling of the veins on the under sides of the affected leaves. Marked phloem necrosis is produced throughout and shows as more or less conspicuous dark rings in cross sections of the root.

The disease is known to occur in only the semiarid regions of the western part of North America.

Curly-top is the cause of greater losses than any other sugar beet disease in this country. Very great damage to beets results when they are grown as an annual crop and when grown for seed production.

The exact nature of the cause of the disease is unknown, but the fact has been demonstrated that the virus can be transmitted by the beet leafhopper (*Eutettix tenella* Baker). No other means of transmission is known. The ability to produce the disease is not inherent in the leafhopper. There is some evidence that an interval of a few hours, an incubation period, must elapse after a nonviruliferous leafhopper has fed on a diseased plant before it can effectively transmit the virus to a healthy plant. Ten or more insects are more likely to produce the disease in an inoculated plant than when the inoculation is made with one or two insects. In cases where the disease is produced there is no difference in the incubation period or severity of the effect between inoculation with single insects and those with ten insects. A viruliferous leafhopper may not produce the disease each time it feeds on a healthy plant, even though the periods of feeding be 24 or 48 hours or even longer.

Tests in regard to the relation of light to infection indicate that plants kept in darkness during inoculation are more readily infected through the cotyledons than through the true leaves.

Tests of the effect of heat on the disease indicate that the virus is not destroyed by temperatures lower than those at which the beet tissue is killed.

Limited data suggest that the virus may be destroyed by desiccation.

The virus is distributed through all parts of an affected plant.

Limited data suggest evidence opposed to the claim that a specific bacterial organism is associated with curly-top.

The incubation period of the disease varies usually from 7 to 14 days. As short an incubation period as 4 days has occasionally been noted.

The incubation and development of the disease is retarded by low temperatures.

Very young plants are more readily infected than are older plants.

A wide range of species has been found susceptible to curly-top.

The virus probably overwinters in susceptible wild annuals, in volunteer beets, and in the insect.

No satisfactory control of the leafhopper by artificial or biological means has been discovered. Early planting usually avoids much of the injury. There is basis for belief that a resistant strain of beets may be developed.

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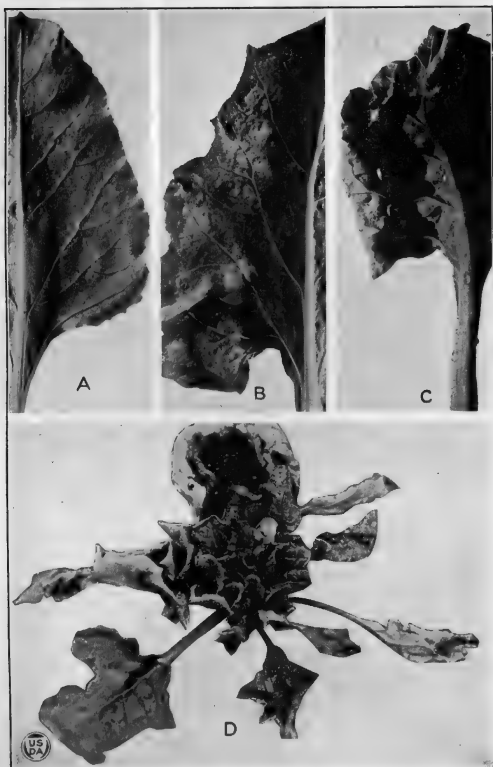
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## PLATE 1

Early stage of curly-top. Note the swelling of the veinlets on the lower surface of one leaf and the irregularity of the upper surfaces and the curled condition of the other young leaves.

(320)





## PLATE 2

A.—A type of curly-top beet leaf. Note the inconspicuous rounded swelling scattered over the surface.

B.—Curly-top beet leaf. Note the general distribution of the moderate, irregular swellings on the smaller veins. This specimen is representative of one type of response to the disease stimulus.

C.—Exudate on a curly-top leaf. The clear, viscid exudate has a sweet taste. Fehling's test reveals the presence of sugars after hydrolysis with acid.

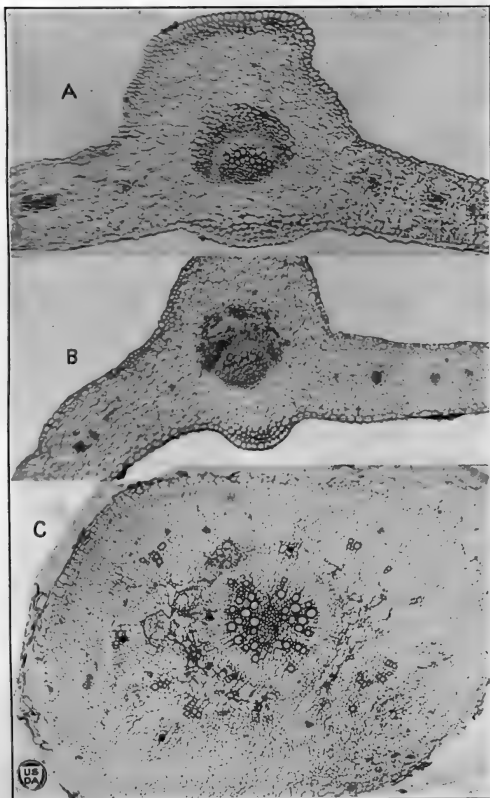
D.—Sugar beet curly-top. Plant inoculated on July 19, 1918, and photographed on August 29, 1918. Note the length of the old leaves, which were about mature when the plant was infected, in contrast with the dwarfed younger leaves.

### PLATE 3

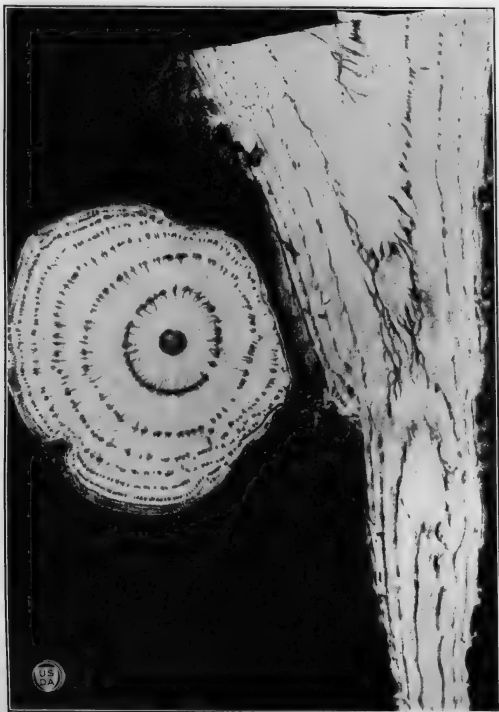
A.—Cross section of lateral vein of a healthy beet leaf.

B.—Cross section of lateral vein of a curly-top beet leaf. Note necrosis in the phloem region.

C.—Cross section of a young, curly-top beet root. Note the dark circle of necrotic phloem. All X about 70.







#### **PLATE 4**

Sections of a curly-top beet root. The phloem necrosis which shows as the darkened tissues is often less conspicuous than in this specimen. X 2.

## PLATE 5

A.—*Calandrinia menziesii* T. & C. Normal plant.

B.—Plant of about the same age but affected with curly-top. From plants diseased, as is *B*, from inoculation with viruliferous insects the disease has been communicated to healthy beets by the use of nonviruliferous leafhoppers.

C.—Resistance to curly-top. The beets in the row on the right were the progeny of a plant selected for resistance. The control row adjacent on the left was from commercial seed. Both rows were exposed to the same natural infestation of leafhoppers, and in addition all the plants of the selected strain had viruliferous leafhoppers caged on them.



# PECAN SCAB WITH SPECIAL REFERENCE TO SOURCES OF THE EARLY SPRING INFECTIONS<sup>1</sup>

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## INTRODUCTION

Owing to the extensive development of the cultivated pecan industry throughout the southeastern United States during the past 15 or 20 years, the economic importance of those diseases which materially decrease production has assumed a prominent position. Excluding rosette, which is apparently caused by, or at least associated with, unfavorable soil conditions, scab caused by *Fusicladium effusum* Wint., is preeminently the most important pecan disease.

## DISTRIBUTION

Though the pecan is indigenous along the several rivers in Texas, the Mississippi, and its tributaries, as far north as central Illinois and southern Indiana, scab is known to exist only in the Southern States bordering the Atlantic Ocean and Gulf of Mexico, and in Arkansas. These facts indicate that high humidity as well as high temperature may be essential for its maximum development.

In the southeastern United States pecan scab was until recent years of serious economic importance only within a distance of 50 to 100 miles from the coast line, but now it seems to be extending its range of distribution inwardly. This condition is thought to be due largely to the increased number of plantings and to the frequent summer rains of the past few years, which have favored the rapid spread of the disease. The apparently rapid spread of scab in the central section of the southern pecan belt<sup>2</sup> may also be accounted for by the fact that the cultivated pecan industry is comparatively new and many orchards are young, having only recently come into bearing. A young orchard composed of one of the very susceptible varieties, such as Delmas, even when situated near the coast, may produce two or three crops of disease-free nuts before the scab fungus has become so well established as to reduce the yield of nuts. Even now the disease is of a less serious nature and fewer varieties are attacked 150 miles from the coasts than at points within 50 miles of them. Certain varieties, such as Van Deman, Schley, and Pabst, are very susceptible when grown near the Gulf and southern Atlantic coasts, but are at present only slightly attacked at points 100 miles from these coasts.

Not considering the natural agencies favoring the spread of pecan scab, the growers in many instances are partly, if not largely, responsible for the presence of the disease in its most accentuated form. This condition is brought about by the occasional neglect of orchards that have become unprofitable due to the loss of the crop by scab. Some growers think there will be a return of weather conditions, totally unfavorable for the development of the disease, which will permit their trees again to become productive. During this interval of neglect,

<sup>1</sup> Received for publication Feb. 27, 1924.

<sup>2</sup> The southern pecan belt is fairly well confined to that portion of the South in which cotton grows.

every leaf, stem, and nut growing on the susceptible trees becomes badly infected and the orchards become public nuisances, spreading the disease to near-by orchards in all directions.

The distribution of pecan scab is very noticeably associated with climatic conditions. With the exception of the humid Coastal Plain region, Texas ordinarily is practically free of the disease. Therefore, in that State, Delmas, one of the most susceptible varieties, is strongly recommended for planting. The disease is most destructive and attacks the widest range of varieties in the humid coastal region of Louisiana, Mississippi, Alabama, Georgia, and Florida.

### VARIETAL SUSCEPTIBILITY

At present there is a wide variation in the degree of susceptibility of the different cultivated pecan varieties to scab. Some varieties are so severely attacked during seasons favorable for the dissemination and propagation of the fungus that the entire crop may be destroyed. Other varieties are so highly resistant that they may at present be considered almost immune. Wild or planted seedlings show the same variation in resistance.

It seems probable that, in some instances, the distribution of pecan scab and apparent differences in the relative susceptibility of the same varieties growing in different localities can be attributed to different strains of the pathogen. If different strains do not exist, then how can one explain why two susceptible varieties, as Delmas and Schley, can grow in contiguous rows for several years, the Delmas variety being badly infected but the Schley free of the disease? Then after an interval of a few years, the Schley also becomes infected, at first only slightly so, but later severely. Possibly, the Schley strain if there is such, was present in the Schley orchard during the time of its apparent absence, but increasing slowly, finally became abundant in the Schley orchard. It is possible that the strain on the variety Schley may have developed from the Delmas strain by some process of adaptation.

A classification of various cultivated varieties of pecans according to the degree of their susceptibility is not easy, in as much as the susceptibility of each variety varies in widely separated localities. What may be considered at present as a highly resistant variety in one locality may be a susceptible one in another place. Generally speaking, however, Delmas is the most susceptible variety followed by the varieties Georgia, Alley, Van Deman, Schley, Pabst, Mobile, Success and Moore, in the order named. The following varieties, while probably not entirely immune, are highly resistant: Teche, Curtis, Moneymaker, Russel, Stuart and Frotcher. The Texas varieties are purposely omitted from the list as ordinarily pecan scab is not an important disease in that State. Some Texas varieties, notably, the San Saba, are highly susceptible when planted in the humid region of the southeast United States.

### DESCRIPTION OF THE DISEASE

#### ON THE LEAVES

On the leaves the infections first appear as elongated spots on the large vein on the underside of the pinnae or on the rachis and petiole, Plate 1, A. A few days later, spots may be noticed on the underside of the smaller veins also. The lesions on the small veins are at first elongated but later diffuse irregularly into the healthy tissues, forming more or less circular spots. Some infections appear to have taken place on the upper surface of the leaflets. Apparently not all spots begin on the veins, since some appear on the green parts lying within the network of the vascular system. The spots are at first olive brown in color, but turn almost black with age. They are quite conspicuous on the leaves and

especially so on the light yellow green of the young leaves. The infections on the leaf may be few or many, depending upon the variety, the abundance of sources of infection and the weather conditions. Leaves become more immune as they reach maturity and as a rule, are only slightly susceptible after they change from the light yellowish-green of the young stage to the later deep green color. The control of the disease would be much simplified if the pecan did not have the habit of putting out new leaves and twigs after the initial spring growth. A heavy second growth of foliage, as a rule, is produced about the 1st of July or soon after the usual summer rains commence. While on the light growth of new leaves during May and June new infections continue to occur thus forming new sources of infection, it is the new infections on the midsummer second growth of leaves which serve greatly to increase these sources of infection. A heavy increase of the disease on the nuts when they are about one-half grown as a rule follows the severe spotting of the second growth of foliage. Ordinarily, the development of the midsummer second growth of foliage extends over a period of four to six weeks. During this time new leaf surfaces are continually being exposed to infection. A protective spray may be applied today but to-morrow new leaflets are exposed and unprotected.

The damage done to the leaves as a whole is of minor importance, though considerable defoliation, especially in nurseries, sometime occurs (Pl. I, G), and heavily infected trees shed their leaves early in the autumn. Several infections on the veins of the ventral side of the leaflets cause them to curl downward as though injured by aphids. The punctures of aphids, often found feeding on the veins during early spring, are considered by Waite<sup>3</sup> as points of entrance for the pathogen. Possibly the greatest damage done to the leaves is due to the interference with their normal functioning caused by the partial girdling of the petioles from numerous infections near their bases.

#### ON THE NUTS

Narrow, elongated spots frequently appear first on the young nuts along the creases which run parallel to the long axis of the nut midway between the sutures of the pericarp, and which correspond morphologically to the veins of the leaflets (Pl. 1, C and D). Lesions are quite small at first and slowly enlarge, some attaining a diameter of about 12 mm. Most spots, however, cease to enlarge after they are about 3 mm. in diameter. The scab spots on the nuts may be either olive-brown or gray when first visible. Those infections which are at first gray later change to an olive-brown with a gray border. All scab spots, regardless of shape or color, possess a more or less jagged, irregular, and indefinite outline.

The greatest damage is done to the nuts, which may become undersized, faulty, or completely destroyed. The diseased areas are rather superficial, extending only slightly below the epidermis. The affected tissues become black, hardened, and somewhat cracked, but show no tendency to decay (Pl. 1, D). It is not uncommon, however, for secondary fungi to gain entrance through the ruptured surface caused by the scab pathogen and produce deep-seated lesions extending down through the exocarp to a depth of 2 to 2½ mm. The secondary fungi apparently do not enter the husk until the nut is from one-half to two-thirds grown. When scab infections occur while the nuts are small, or when numerous infections occur during midsummer, the growth and development of the nuts are completely retarded, and they either fall before attaining their normal size or blacken and hang on the trees all winter (Pl. 1, E; Pl. 2, D). The death of the nuts often seems to be due to the coincidence

<sup>3</sup> WAITE, M. B. PECAN SCAB [*FUSICLADIUM EFFUSUM*]. *Science* 33: 77-78. 1911.

of two factors: First a great number of infections directly causing the death of the nuts; and, second, infection of the pedicels, which are often so severely attacked that the flow of water and nutrient materials is partially or even completely shut off. Frequently the pedicel is killed outright while the nut is yet partly green.

#### ON THE TWIGS

The twigs of certain varieties of the pecan, especially the variety Delmas, are very susceptible to scab. The terminal twigs on old, neglected Delmas trees are often killed back from 7 to 15 cm. in one season by the scab pathogen (Pl. 2, A). The twigs of certain other pecan varieties, such as Alley, Van Deman, and Schley, are also susceptible but to a much lesser degree than those of the Delmas variety. Only the twigs of the current year's growth are attacked by the scab fungus, and these are susceptible only while they are young, growing rapidly, and before lignification takes place. The scab spots on the twigs are olive in color, while the fungus is sporulating, but are black after the spores have been washed away. In size, the lesions range from 0.5 to 3 mm. in diameter (Pl. 2, B and C).

#### ON THE CATKINS AND DORMANT BUDS

The writer has observed scab on pecan catkins only in a few instances. Apparently no injury is done to this floral part as the spots are few in number and have been seen only on the pedicels and bracts. The fungus does not seem to attack the pollen sacs.

It is not unusual to find from one to three scab spots holding over from the previous seasons on a dormant bud. From sections made of such infected buds, the writer is led to believe that mycelium does not extend deeper than the outer bud scale.

#### SOURCES OF PRIMARY INFECTIONS

According to those who have made a thorough study of the apple scab fungus, the conidial stage of which greatly resembles *Fusicladium effusum*, the principle source of early spring infections is the ascogenous stage formed within the tissues of the fallen leaves of the previous season. The writer has frequently observed several forms of ascomycetous fungi on dead pecan leaves, twigs, and nuts, but none has been specifically connected with the pecan scab fungus. While an ascogenous stage, which one would expect to be a *Venturia*, may exist, it is as yet unknown. Many orchard and laboratory observations have led the writer to believe that even should *Fusicladium effusum* produce a perfect stage it would certainly be only a contributory factor in the perpetuation of the fungus, as in the South the conidial stage regularly lives overwinter in great abundance.

#### TWIGS AS SOURCE OF PRIMARY INFECTIONS

Under certain favorable conditions, the hyphae in scab lesions on twigs remains alive during the mild winters of southern Georgia, and the following spring becomes very important sources of the early spring infection. The mycelium does not remain alive overwinter in all twig lesions. The scab spots formed during the early part of the season apparently are not able to live after the cortex of the twig reaches a certain stage of maturity. By the end of the summer, such early formed lesions become flattened and cracked and the fungus within appears to be dead (Pl. 2, C and D). During the following spring many of them are either partially healed over or appear as black, sunken spots with slightly raised rings of cortical tissues surrounding them (Pl. 2, E). In these older spots, resulting from early spring infections, the fungus seldom lives through the winter and they do not, therefore, appear to serve as sources of the primary infections.



Twig lesions formed during the latter part of the summer or early autumn develop black more or less hemispherically shaped, sporulating stromata on their surfaces (Pl. 2, B). The author considers the sporulating stromata thus formed as very important sources of the early spring infections. They have frequently been observed producing conidia abundantly during April and May at the time the new leaves were unfolding and extremely susceptible to scab infection. Pecan twigs infected as illustrated in Plate 2, B, when collected at various times during the winter and spring months and placed in damp chambers for 48 hours, have almost invariably produced conidia from the stromata in great profusion. Spots on twigs that have been killed by a severe attack of scab during the previous season also serve to carry the disease over winter.

Primary infections from such sources as those just described are the most difficult to prevent. Pecan leaves are very susceptible to attack of the scab pathogen during the time they are unfolding. During this period, young pecan leaves are also very susceptible to spray injury, thus increasing the difficulty of preventing early leaf infections. Pecan leaves unfold over a period of several days, during which time an application of spray may do great damage to the foliage and serves only to protect those pinnae already unfolded and exposed.

#### OLD NUTS AS SOURCE OF PRIMARY INFECTIONS

Nuts that have been killed by the scab fungus during the previous summer are, as a rule, important sources of the early spring infections. All such nuts, however, seem not to carry the fungus through the winter, but only those that have attained one-half size or more before being killed by the disease. The nut, illustrated in Plate I, E, is a good example of this type of the primary infection. The dark blotches, seen on the stem-end of such nuts will produce conidia during the early spring weeks. Frequently, nuts are slightly spotted with scab during late summer. Infections taking place at this time may cause no damage to the nuts since the kernel develops and matures normally. These late infections usually form the sporulating stroma and serve as a means of carrying the disease through the winter. Either empty shucks or late season infected nuts are quite noticeable as sources of the early infection when they remain on the trees over winter and become the center of a number of scab-infected leaves the following spring.

#### LEAVES AS SOURCE OF PRIMARY INFECTIONS

Scab spots that have formed on the smaller veins of the leaflets or those that have formed within the network of the vascular system do not form sporulating bodies and are, therefore, not believed to harbor the fungus through the winter. Lesions on the mid-veins of the pinnae, rachises and petioles, especially those that form near the base of the petiole produce the hold-over stroma, and consequently serve as sources of the primary infections.

#### CONTROL MEASURES

During the pioneer days of the cultivated pecan industry, the variety Georgia was thought to be the most susceptible to pecan scab. The growers early realized the seriousness of the disease on this variety and promptly top-worked the Georgia to varieties considered at that time as being resistant or immune. Mistakes were made during this period of varietal changing, owing to the then apparent resistance of some varieties now known to be exceedingly susceptible. For instance, the Georgia variety was top-worked to Delmas. Later, Delmas orchards were top-worked to the variety Schley. The last-named variety is now known to be very susceptible in some localities. While many growers are still discarding the susceptible varieties by top-working to the more resistant ones, this method

of disposing of the disease is less popular than formerly, owing to the apparent adaptation of the fungus to the more resistant ones. Spraying is now being considered somewhat more seriously as a means of reducing the loss by scab, but as yet most of the growers seem to prefer top-working to the more painstaking and labor-involving operation of spraying.

During the past two years the writer, in search of the most satisfactory spray for pecan-scab control, has been devoting considerable attention to trials with different kinds of fungicides commonly used in apple and peach orchards. In the order named, Waite,<sup>4</sup> Spooner,<sup>5</sup> McMurran and Demaree,<sup>6</sup> and Neal<sup>7</sup> have shown Bordeaux mixture to be quite effective as a spray for the control of pecan scab. Because this spray sometimes causes serious injury to the foliage an attempt was made to find some spray that would be effective and yet cause no serious foliage injury. The following fungicides have been used: Sulphur dust, Bordeaux dust, lime sulphur solution, wettable sulphur, self-boiled lime sulphur, lime sulphur modified by the addition of 1 pound of copper sulphate to each 50 gallons of spray and self-boiled lime sulphur modified by the addition of 2 pounds of copper sulphate to each 50 gallons of spray. Both types of dust, wettable sulphur and self-boiled lime sulphur, even when modified with copper sulphate, proved to be ineffective under the conditions tried. Lime sulphur solution when used at the strength of 1 gallon of the concentrated material (32 to 33° B.) to 50 gallons of water, controlled the disease quite satisfactorily during the season of 1922. This spray, however, proved to be much less effective during the rainy summer of 1923. From the results of the work so far conducted, it appears that the choice of a spray will be limited to Bordeaux mixture which is ordinarily effective but may cause serious foliage injury under certain weather conditions, and lime sulphur solution, a spray only slightly injurious to the foliage, but whose capabilities as a preventive of pecan scab are not yet fully demonstrated.

Since it is difficult to control pecan scab even under the most favorable conditions, advantage should be taken of every possible opportunity to reduce in number the sources of infection. As previously mentioned, the unfolding leaves are quite susceptible to spray injury. The foliage is also very susceptible to scab infection during this same period. Therefore, the importance of destroying in so far as possible all the sources of the primary infection is quite evident. The writer has been informed of instances where fire swept through badly infected orchards, burning the debris on the surface of the ground. As a result, it was reported that there was a very pronounced reduction in the amount of the disease in these orchards during the season following. The effect of numerous overwintering infection sources on control by spraying was brought to the writer's attention in a very striking manner last season. An orchard of the variety Schley, at Quincy, Fla., was sprayed on the following dates: April 10, May 9, May 23, June 13, July 5, and 27, 1923. On account of frequent rains during June and July, the spray could not be applied according to any prearranged schedule. No attempt was made to destroy the sources of the primary infections. The soil had not been plowed and the last year's leaves, shucks and mummied nuts were left upon the ground. Consequently, a severe primary infection of the leaves took place very early in the spring, even before it was

<sup>4</sup> WAITE, M. B. NUT DISEASES WITH SPECIAL REFERENCE TO THE PECAN. *Proc. Amer. Pomol. Soc.* 32: 182-190. 1911.

<sup>5</sup> SPOONER, C. S. PECAN SCAB (*FUSICLADIUM EFFUSUM*). *Georgia State Bd. Ent. Bul.* 49: 38-43, illus. 1918.

<sup>6</sup> McMURRAN, S. M., and DEMAREE, J. B. DISEASES OF SOUTHERN PECANS. *U. S. Dept. Agr. Farmers Bul.* 1129, 22 p., illus. 1920.

<sup>7</sup> NEAL, D. C. SPRAYING EXPERIMENTS FOR THE CONTROL OF PECAN SCAB IN MISSISSIPPI. *Miss. Agr. Exp. Sta. Bul.* 203, 14 p., illus. 1921.

thought to be safe to spray on account of probable injury to the tender foliage. The result of this spraying experiment was a complete failure as far as control was concerned. In spite of six applications of spray, of which five were with standard strength Bordeaux mixture, the disease completely destroyed the crop. This orchard was sprayed four times with Bordeaux mixture during the previous season and it is thought that twig infections of that year played a very minor part in causing the primaty infections of the past year.

As a contrast to the Quincy, Fla., experiment, the results are given below of a spraying test at Thomasville, Ga., on the same variety. This orchard had been sprayed for two years previous, and the ground was carefully plowed during late winter so that all leaves, petioles, and shucks were buried under the soil. The disease did not appear in this orchard until about the first of June. An application of spray at this time was highly desirable but was postponed until June 6 on account of rainy weather. The second application was made July 8, and the third and last one August 3. As a result of this work, approximately 89.6 per cent of the crop of the Bordeaux-sprayed trees was saved. The control trees in this orchard matured some good nuts, but 56 per cent of the crop fell before maturity. Of those nuts remaining on the trees until harvest 20 per cent were either totally bad or faulty. The sprayed nuts averaged 64 to the pound, while the unsprayed ones averaged 74. It is not thought that the weather conditions at Thomasville were more favorable for a spraying experiment than at Quincy, Fla., as it was unusually rainy and cloudy during the season. Including days with a trace, rain fell on 19 days in May, 24 days in June, 21 days in July, and 15 days in August at Thomasville. No record of the rainfall at Quincy, Fla., is available.

A standard spray schedule for pecan scab has not yet been definitely established. A spray schedule that produces satisfactory results one season often proves to be entirely ineffective another one. Very satisfactory results were procured at Dewitt, Ga., in 1922 by applying the spray on the following dates: May 1, May 17, June 6, June 28, July 19, and August 19. A similar schedule followed during the season of 1923 in the same orchard did not control the disease satisfactorily. At Baconton, Ga., in 1922, excellent results were obtained by applying spray on the following dates: June 13, July 6, July 27, and August 23. In 1923, despite the fact that spraying in this orchard was begun a month earlier and two more applications were made than in 1922 the results were not satisfactory.

As a general recommendation in the light of our present knowledge and experience, the first application of a protective spray should be made immediately after the nuts have set. Later applications should be made at intervals of two or three weeks. The weather conditions, the amount of scab in the orchard the year previous, the relative susceptibility of different varieties, and the attention paid to sanitation are factors that must govern the number and time of application.

#### DORMANT SPRAY

In an endeavor to eliminate the twig lesions as sources of the early spring infections, Bordeaux mixture containing 8 pounds of bluestone and 8 pounds of stone lime to 50 gallons of water and lime sulphur solution prepared by adding 6 gallons of the concentrated solution (32 to 33° B.) to 42 gallons of water have for three successive years been applied as dormant sprays. The time of application was in all cases delayed until a few days before or even after the buds begin to swell. While it appears that a strong fungicide would kill the hold-over stromata, only slight evidence has been secured by the writer favorable to the use of the winter spray.

## DIFFICULTIES ENCOUNTERED IN ENDEAVORS TO CONTROL PECAN SCAB

Those attempting to control pecan scab encounter several perplexing difficulties. In addition to the problems arising in the control of other deciduous fruit diseases, even more perplexing ones appear. The problem of spraying the tops and centers of some of the larger pecan trees is not an easy one. Despite the youthfulness of the cultivated pecan industry, some of the older cultivated trees have attained the height of 50 to 60 feet with a spread of 40 to 60 feet. The usual tower and spray rods are not practicable to use when spraying trees of such dimensions and resort must be had to the high-power spray outfits and spray guns. Most of the spray guns used by the writer are not capable of carrying the desired misty spray to the tops of the taller trees. It is hoped that horticulturists will devise some system of pruning that will tend to reduce the height of the trees and open up their centers so as to facilitate spraying operations.

The pecan nut is susceptible to the attack of the scab pathogen from the time it is first formed, which is ordinarily during the latter part of April or the first part of May, until it has completed its growth. Any meristematic tissue seems to be subject to infection, necessitating, in case of the very susceptible varieties, frequent applications of spray from the first of May to the middle of August.

Another difficulty in controlling pecan scab is due to the susceptibility of certain varieties such as Delmas to twig infection. Since there is ample evidence that large numbers of spores are produced on infected twigs in the early spring, one can readily see how easily the unfolding leaves situated in such close proximity to these sources of infection may become infected. Twig infections on the current year's growth also serve to propagate the fungus during the summer.

The rainy season through the central and southeastern sections of the pecan belt usually begins about the middle of June, and normally extends over a period of approximately three months. The average number of rainy days for June, July, and August at Thomasville, Ga., according to the United States Weather Bureau is 12, 15, and 14, respectively. Then, normally, rain may be expected on an average of almost every other day during those three months. Occasionally the number of rainy days greatly exceeds the average, as for example, July, 1921, had 27; July, 1922, 18; June, 1923, 24; and July, 1923, 21.<sup>8</sup> It is often difficult and occasionally impossible, on account of boggy soil, to apply spray at the times one thinks it should be put on during June, July, and August in the Coastal Plain region.

Coincident with the rainy period, the pecan trees put on a new growth of twigs and foliage, which greatly complicates spraying operations on account of the extreme susceptibility of the new foliage.

## SUMMARY

Excluding rosette, pecan scab, caused by *Fusicladium effusum* Wint., is the most important disease affecting the pecan.

The disease is known to exist in all the Southern States bordering the Atlantic Ocean and Gulf of Mexico and in Arkansas.

At the present time there is known to be a wide variation in the degree of susceptibility of the different cultivated pecan varieties to the scab disease. Some varieties are very susceptible while others are almost immune.

The disease is known to attack the nuts, twigs, leaves, dormant buds, and catkins. Very little, if any, damage is done to the buds and catkins. Consider-

<sup>8</sup> The Weather Bureau indicates a rain measuring less than 0.01 of an inch as a trace. Days having only a trace of rain are included in the number of rainy days herein reported. The author feels justified in including the days when the precipitation indicates only a trace, as a misty, cloudy day, when the actual precipitation measures less than 0.01 of an inch may be much more favorable to spore germination and infection than a heavy shower followed by bright sunshine.

able defoliation, especially in nurseries, sometimes occurs. Severely infected trees usually shed their leaves earlier during the fall than they normally should. Occasionally the tender tips of the twigs are killed back from 5 to 15 cm. The greatest damage is done to the nuts which may become undersized, faulty, or rendered entirely worthless.

An ascogenous stage of the pecan scab fungus is not known to exist. The fungus lives through the winter months in the form of more or less hemispherically shaped stromata situated in lesions upon the surface of twigs, rachises, petioles, and shucks. These stromata produce conidia the following spring.

Hygienic measures tending to eliminate, as completely as possible, the various sources of the early spring infections, are very important as a means of keeping the disease within bounds so that spraying may be made effective. Twig lesions can be largely prevented by thorough summer spraying. The most practical method of disposing of the infected leaves and shucks after they fall to the ground is the thorough plowing under of these parts during late winter. If practicable to do so, the raking and burning of these affected parts would also be effective.

Of the various available spray materials tried Bordeaux mixture was the most effective, although it sometimes causes serious injury to the foliage.

Strong Bordeaux mixture and strong lime sulphur solution used as dormant sprays did not perceptibly reduce the amount of infection.

The following are mentioned as instrumental in making effective control of pecan scab difficult: The large size of the pecan trees to be sprayed; the normal frequency of summer rains prevailing in the southern pecan belt during June, July, and August; the long season of susceptibility of the pecan nut to scab infection; the close proximity of the young leaves to hold-over stromata on the twigs; and production of a second growth of leaves and twigs, subject to infection if not protected by spray.

## PLATE 1

A.—Pecan leaf showing various types of infection of pecan scab. The photograph was taken especially to show the elongated lesions of the midrib of the pinnae.

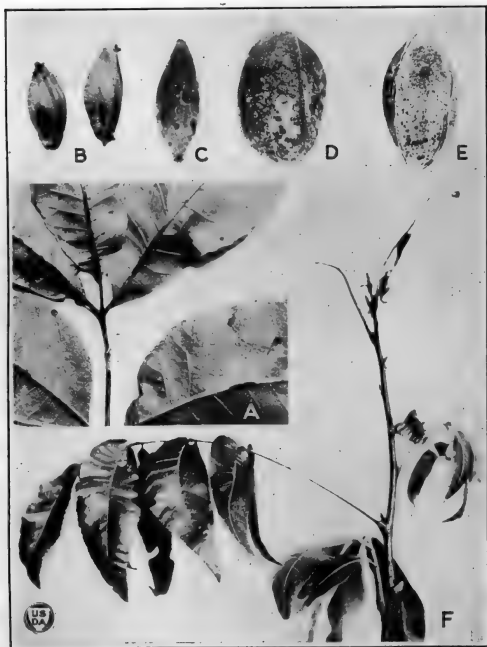
B.—Young pecan nuts showing elongated lesions situated lengthwise along the center of the segments of each nut. About natural size.

C.—Pecan scab lesions as they appear irregularly over the surface of the nut. About natural size.

D.—A pecan nut about one-half developed, showing very numerous scab infections. Three-fourths natural size.

E.—A pecan nut killed during midsummer, due to a heavy infection of pecan scab. The dark blotches on the stem-end of the nut are hold-over stromata. Such nuts usually fall to the ground before harvest, but some remain attached to the twigs all winter.

F.—A pecan twig taken from a nursery showing premature defoliation due to pecan scab. Photograph taken July 17, 1920.







## PLATE 2

A.—A pecan twig killed during the year previous by a severe infection of pecan scab.

B.—The black spots on the middle portion of the twigs are slightly raised masses of closely woven mycelium formed by the pecan scab fungus. Such spots produce *Fusicladium* spores during early spring. About natural size.

C.—The large flat lesions on the middle portion of the twig are the results of early spring infections. The fungus is dead in such spots and does not form the overwintering stromata. Natural size.

D.—A last year's scab-killed nut, situated among the new leaves and inflorescence. Scab-killed nuts are often a fertile source of the early spring infections.

E.—A pecan twig showing old scab spots that are now beginning to heal over.

# ANTHELMINTIC EFFICIENCY OF CARBON TETRACHLORID IN THE TREATMENT OF FOXES<sup>1</sup>

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Investigations conducted by the Bureau of Biological Survey of the United States Department of Agriculture tend to indicate that many foxes, both in the wild and on ranches, are infested with hookworms, principally *Uncinaria stenocephala* and occasionally *Ancylostoma caninum*. Although many foxes harboring hookworms manifest no apparent symptoms of infestation, observations made indicate that heavy infestations may be attended with impaired health and may even result in death.

In view of the fact that Hall (2)<sup>3</sup> found chemically pure carbon tetrachlorid in sufficient doses to be relatively effective and safe in the treatment of dogs for the removal of hookworms, it was considered advisable to test this drug as an anthelmintic for foxes. The results of the experiments conducted are reported in this paper.

## ANTHELMINTIC EXPERIMENTS

In the experiments on foxes, the technic was similar to that used by Hall alone or in collaboration with other investigators (2, 3). Chemically pure carbon tetrachlorid was given in hard gelatine capsules or in soft elastic globules to foxes that had been fasted from 16 to 20 hours. The only animals which were not fasted the usual period were foxes No. 792 and 793, two 1-month-old nursing pups. No purgative was given before or after the administration of the dose, except to fox No. 88.

After treatment each animal was confined in a small cage which was provided with a heavy gauge 1-inch woven-wire bottom and set on supports, with a large galvanized iron collecting pan underneath to catch all feces and worms passed. In the use of cages of this type it was found that the number of worms eaten or adhering to the feet or fur of the foxes was reduced to a minimum. The feces and worms passed were collected daily, screened, and all worms passed were identified and counted.

From three to five days after treatment the animals were killed with either a subcutaneous or an intrathoracic injection of strychnin sulphate solution. A post-mortem examination was made of each animal to determine the kind and number of worms remaining in the alimentary tract; also to make note of the gross pathological changes, especially those apparently due to the drug. The percentage efficacy of the treatment in all cases was the figure obtained by ascertaining the relation of the number of worms passed to the combined total of the number passed after treatment and the number found on post-mortem.

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<sup>2</sup> Dr. H. L. Van Volkenberg resigned March 31, 1923, to work under the Bureau of Fisheries on the diseases and parasites of blue foxes on the Pribilof Islands, Alaska.

<sup>3</sup> Reference is made by number (*italic*) to "Literature cited," . 337.

A dose rate of 0.1 cc. per kilo was given in hard gelatine capsules to the following:

*Fox No. 201.*—Weight 5.24 kilos; passed no worms. Post-mortem fourth day, 12 hookworms, 2 ascarids, and 1 tapeworm; 0 per cent vs. hookworms, ascarids, and tapeworms. No apparent symptoms or gross lesions due to the treatment.

*Fox No. 209.*—Weight 2.5 kilos; 19 hookworms first day, 3 hookworms second day; retained no worms; 100 per cent vs. hookworms. Post-mortem fourth day; liver slightly swollen, with tissues bulging slightly on section; region of congestion between cortex and medulla of kidneys; slight congestion of mucosa of small intestine.

A dose rate of 0.2 cc. per kilo was given in hard gelatine capsules to the following:

*Fox No. 203.*—Weight 5.45 kilos; passed 18 hookworms first day; retained no worms; 100 per cent vs. hookworms; no apparent symptoms or gross lesions.

*Fox No. 204.*—Weight 6.36 kilos; passed 7 hookworms first day. Post-mortem fourth day; 1 hookworm and 2 ascarids; 88 per cent vs. hookworms and 0 per cent vs. ascarids; no apparent symptoms or macroscopic lesions due to the treatment.

*Fox No. 786.*—Weight 4.1 kilos; 4 hookworms and 21 ascarids first day; 3 hookworms second day; 2 ascarids third day; killed fourth day and 1 ascarid found; 100 per cent vs. hookworms and 92 per cent vs. ascarids. No apparent symptoms or gross lesions.

*Fox No. 787.*—Weight 3.4 kilos; 11 hookworms first day; 1 hookworm second day; retained no worms; 100 per cent vs. hookworms; no apparent symptoms. Post-mortem fourth day; slight congestion of mucosa of duodenum; liver darker in color than normal, with tissues friable and bulging slightly on section; spleen-swollen.

*Fox No. 788.*—Weight 3.2 kilos; one ascarid first day; killed fourth day and no worms recovered. Post-mortem: Slight congestion of the mucosa of the posterior region of the small intestine; liver light in color and tissues friable; spleen dark and swollen.

A dose rate of 0.25 cc. per kilo was given in soft elastic globules to the following:

*Fox No. 332.*—Weight 4.3 kilos; one hookworm first day; four hookworms second day; killed third day and no worms retained; 100 per cent vs. hookworms; no apparent symptoms. Post-mortem: Tissues of kidney lighter in color than normal and bulging slightly on section; liver slightly darker in color than normal; mucosa of stomach and duodenum congested; wall of duodenum thickened.

*Fox No. 334.*—Weight 4.1 kilos; 3 ascarids and 8 intestinal flukes first day; 1 hookworm second day; killed third day and 2 hookworms present; 33 per cent vs. hookworms and 100 per cent vs. ascarids and intestinal flukes. No apparent symptoms or gross lesions due to the drug.

*Fox No. 356.*—Weight 1.86 kilos; age 3 months; 43 hookworms and 24 ascarids first day; 7 hookworms and 1 ascarid second day; killed third day and 3 hookworms and 1 ascarid recovered; 94.3 per cent vs. hookworms and 96.1 per cent vs. ascarids. Post-mortem: Liver greatly enlarged and darker in color than normal; tissues of kidney bulged slightly on section; region of congestion between cortex and medulla of kidney; mucosa of duodenum slightly congested; wall of duodenum thickened.

A dose rate of 0.3 cc. per kilo was given either in soft elastic globules or hard gelatine capsules to the following:

*Fox No. 88.*—Weight 2.38 kilos; age, 3¼ months; subject to a disease characterized by convulsions; dose given in hard gelatine capsules and followed in 4 hours by 5 cc. of castor oil in capsules; 290 hookworms (*280 Uncinaria stenocephala* and 10 *Ancylostoma caninum*) first day: Fifth day convulsions reappeared and animal died; retained three hookworms; 99 per cent vs. hookworms. Post-mortem: Liver and kidneys congested; brain edematous; petechiae and ecchymosis of small intestine; ecchymotic and suggestion hemorrhages of the cecum and colon; doubtful if carbon tetrachlorid was a factor in the cause of death.

*Fox No. 197.*—Weight 3.4 kilos; drug in hard gelatine capsule; passed 13 hookworms first day; killed fourth day and no worms present; no apparent symptoms. Post-mortem: Liver slightly enlarged; mucosa of stomach slightly congested; few small scattered foci of necrosis of the mucosa of stomach; mucosa of small intestine slightly congested.

*Fox No. 198.*—Weight 3.63 kilos; drug in hard gelatine capsule; passed 13 hookworms and 1 ascarid first day; killed fourth day and no worms present; 100 per cent vs. hookworms and ascarids; no apparent symptoms or gross lesions due to treatment.

*Fox No. 200.*—Weight 2.7 kilos; drug in hard gelatine capsule; no worms found in feces or upon autopsy examination; no conclusions as to efficacy; no apparent symptoms. Post-mortem fourth day: Slight congestion of the kidneys and mucosa of duodenum.

*Fox No. 333.*—Weight 3.3 kilos; drug in soft elastic globule; passed two hookworms and two intestinal flukes first day; killed third day and no worms present; 100 per cent vs. hookworms and intestinal flukes; no apparent symptoms; mucosa of stomach and small intestine slightly congested.

*Fox No. 335.*—Weight 3.1 kilos; drug in soft elastic globule; passed two hookworms first day; no worms present when killed third day; 100 per cent vs. hookworms; no apparent symptoms. Post-mortem: Liver darker in color than normal and blood oozed on section; tissues of kidney bulged slightly on section; mucosa of duodenum congested and wall thickened; Peyer's patches prominent.

*Fox No. 357.*—Weight 1.7 kilo; age 3 months; drug in soft elastic globule; 10 hookworms and 15 ascarids first day; 1 ascarid second day; killed third day and 4 hookworms found; 71.4 per cent vs. hookworms and 100 per cent vs. ascarids; no apparent symptoms. Post-mortem: Liver icteric and light in color with tissues friable; mucosa of small intestine slightly congested.

*Fox No. 792.*—Weight 0.68 kilo; age 34 days; length of fast not known, because this unweaned pup was removed from vixen and treated 1 hour later; drug in soft elastic globule; passed 15 ascarids first day; died second day after treatment; no worms present on autopsy; 100 per cent vs. ascarids, symptoms of distress, lassitude, weakness, anorexia, and marked constipation became apparent 20 hours after treatment. Constipated feces were reddish in color. Post-mortem: Liver light in color and icteric; lobules of liver distinctly visible, being yellow at periphery and a bright red at center; suggulation hemorrhages of the endocardium of left ventricle; pericardium contained an excess of fluid; mesenteric lymph glands enlarged and edematous, inflammation of the mucosa of jejunum and ileum, contents of large intestine and posterior region of small intestine constipated and hemorrhagic.

*Fox No. 793.*—Weight 0.8 kilo; age 35 days; length of fast not known because this unweaned pup was removed from its vixen and treated 1 hour later; drug given in soft elastic globule; passed 14 ascarids first day; died two days after treatment and 1 ascarid recovered; 93 per cent vs. ascarids; constipation, distress, lassitude, and anorexia became apparent day after treatment; feces passed with worms were hemorrhagic. Post-mortem: Suggulation hemorrhages of the endocardium of the left ventricle; ecchymotic hemorrhages of the lungs; liver light in color and icteric; lobules of liver distinctly visible, being yellow at the periphery and bright red at the center; stomach catarrhal; small intestine inflamed, with evidence of hemorrhage into lumen of posterior region; contents of cecum and colon constipated and hemorrhagic.

A dose rate of 0.35 cc. per kilo was given in soft elastic globules to the following:

*Fox No. 348.*—Weight 2.86 kilos; 17 hookworms first day; killed second day and no worms retained; 100 per cent vs. ascarids; no apparent symptoms or gross lesions due to the drug.

*Fox No. 355.*—Weight 1.4 kilos; age 3 months; 74 hookworms and 36 ascarids first day; 1 ascarid third day; killed fourth day; 1 ascarid retained; 100 per cent vs. hookworms and 97 per cent vs. ascarids; no apparent symptoms. Post-mortem: Liver light in color and icteric, with tissues friable; pericardium contained a slight excess of fluid; mucosa of duodenum slightly congested.

A dose rate of 0.4 cc. per kilo was given in soft elastic globules to the following:

*Fox No. 728.*—Weight 4.54 kilos; affected with sarcoptic mange, but in good physical condition otherwise; 12 hookworms first day; killed fourth day; no worms retained; 100 per cent vs. hookworms; no apparent symptoms. Post-mortem: Mucosa of stomach and small intestine slightly congested; wall of small intestine thickened; region of congestion between cortex and medulla of kidneys.

*Fox No. 731.*—Weight 4.1 kilos; no feces passed in first two days; one hookworm and two ascarids third day; killed fourth day and no intestinal worms present; 100 per cent vs. hookworms and ascarids; no apparent symptoms. Post-mortem: Congestion of liver and pancreas; kidneys darker in color than normal, with region of congestion between cortex and medulla; mucosa of small intestine congested; considerable mucus and fibrin adhered to mucosa of small intestines; heart muscle pale.

A dose rate of 0.45 cc. per kilo was given in soft elastic globules to the following:

*Fox No. 729.*—Weight 3.64 kilos; affected with sarcoptic mange, but apparently in good physical condition otherwise; 18 hookworms first day; 8 hookworms second day; killed third day and 1 hookworm found; 96 per cent vs. hookworms; no apparent symptoms. Post-mortem: Mucosa of stomach and small intestine slightly congested, kidneys darker in color than normal, with region of congestion between cortex and medulla.

*Fox No. 730.*—Weight 3.7 kilos; affected with sarcoptic mange, but apparently in good condition otherwise; 169 hookworms, 1 ascarid, and 2 immature *Physaloptera* first day; killed third day, and no worms retained; 100 per cent vs. hookworms and ascarids; and probably 100 per cent vs. *Physaloptera*; no apparent symptoms. Post-mortem: Liver and kidneys darker in color than normal; region of congestion between cortex and medulla of kidney; wall of small intestine thicker than normal.

A dose rate of 0.5 cc. per kilo was given in soft elastic globules to the following:

*Fox No. 325.*—Weight 3.8 kilos; four hookworms and one ascarid first day; killed fifth day and no worms found; 100 per cent vs. hookworms and ascarids; no apparent symptoms. Post-mortem: Liver darker in color than normal and swollen, with blood oozing on section; slight congestion of mucosa of small intestine.

*Fox No. 500.*—Weight 3 kilos; age 4 months; 31 hookworms and 70 ascarids first day; retained 2 ascarids; 100 per cent vs. hookworms and 97 per cent vs. ascarids. Animal became constipated and died 30 hours after treatment. Weakness and restlessness became apparent an hour or so before death. Post-mortem findings were: Abdominal cavity filled with dark unclotted blood, which turned bright red upon

exposure to air; this blood failed to clot upon standing over 24 hours; suggulation hemorrhages of the endocardium of the left ventricle, the lungs were pale and anemic with a few foci of ecchymosis present; the liver was icteric and its tissues friable; lobules of liver distinctly visible, being bright red at the center and yellow at the periphery; pancreas congested; tissues of the mesenteric lymph glands were hemorrhagic; the mesentery and diaphragm showed suggulation hemorrhages; mucosa of stomach and small intestine congested; wall of small intestine thickened; mucus and shreds of fibrin adhered to mucosa of the posterior half of the small intestine.

A dose of 0.6 cc. per kilo was given either in hard gelatine capsules or soft elastic globules to the following:

*Fox No. 202.*—Weight 4.3 kilos; dose in hard gelatine capsules; collapse and cessation of respiration occurred when the drug was inhaled as a result of one of the capsules dissolving in the mouth before being swallowed; artificial respiration performed and the animal recovered; manifested anorexia and constipation for two days; no worms found in feces or on autopsy examination; no conclusions as to efficacy; killed fifth day. Post-mortem: Liver enlarged; region of congestion between cortex and medulla of kidneys; mucosa of stomach and small intestines slightly congested.

*Fox No. 205.*—Weight 5.45 kilos; dose in hard gelatine capsules; six hookworms first day; killed fifth day; few larval ascarids present which apparently reached the intestines after treatment; 100 per cent vs. hookworms; no apparent symptoms. Post-mortem: Liver congested and tissues friable; region of congestion between cortex and medulla of kidneys; moderate congestion of mucosa of small intestine.

*Fox No. 323.*—Weight 3 kilos; dose in soft elastic globules; two hookworms first day; killed fifth day and no worms present; 100 per cent vs. hookworms; fed 2 hours after dosing and vomited 1 hour later. Post-mortem: Liver dark in color and several irregular white areas (apparently areas of necrosis) present on surface and in parenchyma; tissues of liver friable; tissues of kidney bulged slightly on section; mucosa of small intestine slightly congested and wall thickened.

*Fox No. 326.*—Weight 3.5 kilos; dose in soft elastic globules; 11 hookworms first day; killed fifth day and no worms present; 100 per cent vs. hookworms; no apparent symptoms. Post-mortem: Liver dark in color with tissues friable and blood oozing on section, tissues of kidney bulged slightly on section; pancreas congested and a few petechiae present; mucosa of duodenum greatly congested.

## DISCUSSION

The anthelmintic efficiency of the various dose rates of carbon tetrachlorid used in the above experiments may be summarized as follows:

**AT 0.1 CC. PER KILO.**—In hard gelatine capsules given to two foxes, this dose removed 22 hookworms and left 12, an efficacy of 64.7 per cent; removed no ascarids and left 2, an efficacy of 0 per cent; removed no tapeworms and left 1, an efficacy of 0 per cent.

**AT 0.2 CC. PER KILO.**—In hard gelatine capsules given to five foxes, this dose removed 44 hookworms and left 1, an efficacy of 97.7 per cent; removed 24 ascarids and left 3, an efficacy of 89 per cent.

**AT 0.25 CC. PER KILO.**—In soft elastic globules given to three foxes, this dose removed 56 hookworms and left 5, an efficacy of 91.8 per cent; removed 28 ascarids and left 1, an efficacy of 96.5 per cent; removed 8 intestinal flukes and left none, an efficacy of 100 per cent.

**AT 0.3 CC. PER KILO.**—In hard gelatine capsules or soft elastic globules given to nine foxes, this dose removed 330 hookworms and left 7, an efficacy of 97.9 per cent; removed 46 ascarids and left 1, an efficacy of 97.8 per cent; removed 2 intestinal flukes and left none, an efficacy of 100 per cent. Administered in hard gelatine capsules to four foxes, this dose removed 316 hookworms and left 3, an efficacy of 99 per cent; removed 1 ascarid and left none, an efficacy of 100 per cent. Given in soft elastic globules to five foxes, this dose removed 14 hookworms and left 4, an efficacy of 77.7 per cent; removed 45 ascarids and left 1, an efficacy of 97.8 per cent; removed 2 intestinal flukes and left none, an efficacy of 100 per cent.

**AT 0.35 CC. PER KILO.**—In soft elastic globules given to two foxes, this dose removed 91 hookworms and left none, an efficacy of 100 per cent; removed 37 ascarids and left 1, an efficacy of 97.3 per cent.

**AT 0.4 CC. PER KILO.**—In soft elastic globules given to two foxes, this dose removed 13 hookworms and left none, an efficacy of 100 per cent; removed 2 ascarids and left none, an efficacy of 100 per cent.

AT 0.45 CC. PER KILO.—In soft elastic globules given to two foxes, this dose removed 195 hookworms and left 1, an efficacy of 99.4 per cent; removed 1 ascarid and left none, an efficacy of 100 per cent; removed 2 immature *Physaloptera* and left none, probably an efficacy of 100 per cent.

AT 0.5 CC. PER KILO.—In soft elastic globules given to two foxes, this dose removed 35 hookworms and left none, an efficacy of 100 per cent; removed 71 ascarids and left 2, an efficacy of 97.2 per cent.

AT 0.6 CC. PER KILO.—In hard gelatine capsules or soft elastic globules given to four foxes, this dose removed 19 hookworms and left none, an efficacy of 100 per cent.

In agreement with the findings of Hall (2) and Allen (1), these experiments indicate that administration of carbon tetrachlorid is attended with a relatively high degree of efficacy against hookworms and ascarids. They indicate also that this drug is effective against intestinal flukes and *Physaloptera*, but that it is ineffective against tapeworms.

On the whole, the drug was found about as effective in soft elastic globules as in hard gelatine capsules. In the case of foxes No. 334, 356, and 357, feed and water were given about  $1\frac{3}{4}$  hours after treatment. It is very probable that the dose had not left the stomach and that the feed and water tended to dilute and mask the drug, thus reducing the efficacy of the treatment. Observations made later by the writers but reported in a previous paper (6) tended to indicate that feed and water probably should be withheld from foxes for about three hours after the administration of the drug in soft elastic globules, in order to allow sufficient time for the dose to escape from the globules and leave the stomach.

In two instances (foxes No. 200 and 202) no worms were found either in the feces or on post-mortem, although a microscopic examination of the feces just before treatment had shown the presence of hookworm eggs. Hall (2) reported having had the same experience. The chances are that both animals were infested with hookworms and that the treatment was 100 per cent efficient in both cases.

In another case (fox No. 730) two immature *Physaloptera* were passed and none was present on post-mortem. There is a possibility that this animal harbored more of these worms, which infest the stomach, but which were destroyed and digested to such an extent that they were unrecognizable when passed in the feces. According to Hall and Schillinger (4), worms infesting the stomach are liable to be destroyed by carbon tetrachlorid and digested instead of being voided in the feces.

Of the 13 foxes which were given carbon tetrachlorid in hard gelatine capsules, one collapsed from inhalation of the drug, but under artificial respiration, recovered. On the other hand, none of the 18 animals which were given the drug in soft elastic globules suffered inhalation-collapse. The writers (6) have found that in administering the drug cases of inhalation-collapse were not so likely to be encountered in the use of soft elastic globules as in the use of hard gelatine capsules.

In the above experiments, four of the animals died, but carbon tetrachlorid apparently was responsible for the death of only three. Since both of the 1-month-old pups died, the indications are that carbon tetrachlorid at a dose rate of 0.3 cc. per kilo is not tolerated by nursing pups. The constipation which occurred in these two animals undoubtedly resulted in increased absorption of the drug. Probably there were two other factors which predisposed them to intoxication, one being age susceptibility and the other the diet, which consisted of the milk of the vixen. The few analyses which have been made of the milk of fox vixens indicate that it is very rich in fat. Lamson et al. (8) found that dog puppies were more susceptible to carbon tetrachlorid than full grown

dogs and that the administration of the drug after giving digestible fatty substances such as cream increases the degree of intoxication.

Fox No. 500 manifested lesions which were more or less characteristic of those observed in foxes dying from 1 to 5 days after treatment. The writers have elsewhere (6) called attention to the fact that although many foxes tolerate large doses or repeated treatment, a loss of approximately 4 per cent was suffered from what may be called a delayed intoxication. The doses which these foxes received ranged from 0.3 cc. to 0.54 cc. per kilo. In the majority of these fatalities the dose varied but slightly from 0.4 cc. per kilo. It should be mentioned, however, that many foxes were dosed at the above range of rates and manifested no apparent unfavorable symptoms. Our experience has been that tolerance for carbon tetrachlorid varies not only in different foxes but in the same fox at different times.

Although pathological changes seemed to appear more constantly in the use of larger doses, the indications are that other factors than the dosage are involved in absorption of the drug. Some of these factors probably are: (a) Intestinal stasis, sometimes following treatment with the drug, as reported by Allen (1); (b) diseases, especially those associated with an acute enteritis; (c) character of the feed before and after treatment; and (d) idiosyncrasy of some foxes to the drug.

Even though most adult foxes and pups over 3 months old will tolerate relatively large doses of the drug, it is probably advisable to make a practice of restricting the dosage to a range of 0.2 cc. to 0.3 cc. per kilo. Reasons for this are: (a) This range is usually attended with a relatively high anthelmintic efficacy against the more common intestinal worms of foxes; (b) the danger of systemic absorption of the drug probably tends to vary according to the amount of the drug used; and (c) reinfestation with hookworms and ascarids, especially the former, usually occurs within a few weeks, a factor frequently requiring a repetition of treatment.

In conjunction with carbon tetrachlorid it is undoubtedly advisable to use a satisfactory purgative. The senior author has been conducting tests in which foxes were given a solution of Epsom salts by means of a stomach tube about two hours after the administration of the carbon tetrachlorid in soft elastic globules. Not giving the salts until two hours after such administration of the drug overcomes the possibility of the purgative leaving the stomach before the globules dissolve and release their contents. This method of treatment has been found effective in reducing losses from intoxication, but since it requires catching and dosing the foxes twice in connection with each treatment, a more practical method would be either to use soft elastic globules containing both carbon tetrachlorid and a satisfactory purgative or to administer carbon tetrachlorid and a saturated solution of Epsom salts at the same time by means of a stomach tube. Recent papers by Lambert (7) in the treatment of human patients, and by Hall and Shillinger (5) in the treatment of dogs, indicate that carbon tetrachlorid is best administered with a saturated solution of Epsom salts given at the same time, the drug being safer and the efficacy equally high.

### CONCLUSIONS

Carbon tetrachlorid in doses of 0.2 cc. or more per kilo was found very effective in the removal of hookworms and ascarids from foxes. At a dose rate of 0.25 cc. or more per kilo, the drug was 100 per cent efficient against intestinal flukes.

The drug proved about as effective in soft elastic globules as in hard gelatine capsules; that is, when feed and water were not given until three hours after the treatment.

Although many foxes will tolerate very large doses of carbon tetrachlorid, indications are that it is not advisable to use doses in excess of 0.3 cc. per kilo.

One-month-old fox pups do not tolerate carbon tetrachlorid alone at a dose rate of 0.3 cc. per kilo.

Carbon tetrachlorid, like most anthelmintics, should be used with caution on sick animals.

The use of a satisfactory purgative, such as a saturated solution of Epsom salts, is advised in conjunction with the administration of carbon tetrachlorid to foxes.

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# THE CHEMICAL COMPOSITION OF EDIBLE VISCERA FROM MEAT-PRODUCING ANIMALS <sup>1</sup>

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A study of the nutritive value of the edible viscera of meat-producing animals has been in progress in these laboratories for some time. Results pertaining to the antineuritic vitamin content of these organs have already been published by one of us (?),<sup>2</sup> while subsequent papers will probably deal with their value as sources of the other vitamins and with the biological value of their proteins. The present paper is concerned with the chemical composition of the edible viscera, upon which subject there seems to be but little information in the literature.

The data which are here contributed represent the results of fairly extensive analyses of livers and of less extensive analyses of hearts, brains, kidneys, tongues, pancreas, spleens, lungs, and stomachs from the principal types of meat-producing animals, namely, cattle, hogs, and sheep. In some cases separate analyses were made of a number of specimens of the given organ, while in other cases the analyses were made upon composite samples made up from a number of individual organs.

## SOURCE AND PREPARATION OF ANALYTICAL MATERIAL

The animals supplying the experimental material were selected from among average fat steers, butcher hogs, medium weight veal calves, and fat lambs that were being slaughtered at local abattoirs.<sup>3</sup> The organ to be studied was removed from the slaughtered animal and chilled, and when thoroughly cold it was transported directly to the laboratory, where, after removal of extraneous fat and connective tissue, it was passed several times through a meat chopper and the ground material thoroughly mixed to form a homogenous sample for analysis. The determinations of such constituents as are subject to change through enzymatic or bacterial action were begun immediately and carried on with dispatch to a point where no further change could occur. The other determinations were begun at convenience within a day or two, the sample being stored in the interim in a closed glass container at a temperature of 34° F. Analytical work was generally begun within 24 hours after the slaughter of the animal.

## METHODS OF ANALYSIS

The analytical methods employed have for the most part been amply described in the literature and had previously given satisfactory results in the hands of the authors. Appropriate references to or descriptions of these methods are given below.

*Moisture.*—Moisture was determined by freezing a weighed portion of the sample and drying it to constant weight in vacuo over sulphuric acid.

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<sup>1</sup> Received for publication Mar. 6, 1924.

<sup>2</sup> Reference is made by number (*italic*) to "Literature cited," p. 346.

<sup>3</sup> Valuable assistance in the selection of these animals was rendered by Dr. H. K. Walter, of the Bureau of Animal Industry, inspector in charge of meat inspection at Washington, D. C., to whom the authors acknowledge their indebtedness.

*Ether extract.*—The residue remaining from the moisture determination was pulverized and extracted with absolute ether in a Soxhlet apparatus. The extracted material was weighed directly in the extraction flask after the ether had been driven off and the residue had been brought to constant weight at 100° C.

*Total nitrogen.*—Total nitrogen was determined by the Kjeldahl-Gunning method.

*Purin nitrogen.*—Purin nitrogen was determined by the method of Krüger and Schittenhelm (9) as modified by Fellenberg (3).

*Glycogen.*—Glycogen was determined by the method of Pflüger as described by Grube (5).

*Dextrose.*—Dextrose was determined by the method of Hoagland (6).

*Preparation of cold-water extract.*—The cold-water extracts were prepared by macerating 50 grams of sample with a convenient amount of water, and washing the mixture into a volumetric flask with sufficient water to bring the final volume to 1,000 cc. The suspension thus obtained was shaken about eight times in the course of 24 hours and filtered through a dry, folded filter. The water employed in preparing the extract was cooled before used to a temperature of 2° C., and the operations described were conducted in a cold-storage room maintained at the same temperature. The filtered extract served for the determination of total creatinin and of inorganic phosphorus.

*Total creatinin.*—Total creatinin was determined in 200 cc. of the cold-water extract by the Folin method (4).

*Inorganic phosphorus.*—Inorganic phosphorus was determined in 200 cc. of the cold-water extract by the method of Chapin and Powick (2), "Modification B" of this method being employed.

*Total phosphorus.*—Total phosphorus was determined essentially by an adaptation of the Lorenz (10) method. When other ash constituents were not determined, the procedure was identical with that which has been described elsewhere (8) in connection with the analysis of muscular tissue. When a more or less complete ash analysis was made, an aliquot of the solution obtained from the wet combustion of a much larger sample was employed for this determination.

*Preparation of solution for determination of ash constituents.*—For the determination of the ash constituents, 100 grams of the prepared sample were digested with a nitric-sulphuric acid mixture in a Kjeldahl flask, care being taken to employ minimal quantities of sulphuric acid. As a rule a satisfactory combustion could be obtained by employing about 150 cc. of fuming nitric acid, added in small portions, and 10 cc. of concentrated sulphuric acid. After complete destruction of the organic matter, the clear solution was diluted to a volume of 250 cc. in a volumetric flask, and a 10 cc. aliquot was removed for the determination of total phosphorus by the method indicated. The main portion of the solution, together with washings from the pipette, was transferred to a 400 cc. beaker and evaporated, the residue being heated cautiously until the free sulphuric acid had been volatilized. The material was then dissolved in 5 cc. of concentrated hydrochloric acid and diluted with water to a volume of 100 cc. (Solution A.)

*Copper.*—When copper was determined, Solution A was heated to incipient boiling and saturated with hydrogen sulphid in an Erlenmeyer flask, which was then stoppered and allowed to stand over night. The copper sulphid was then filtered off, washed with hydrogen-sulphid water, and used for the determination of copper by Lowe's iodometric method (1, p. 79).

*Separation of metals and alkaline earths from alkalies.*—The filtrate from the copper sulphid was boiled to expel hydrogen sulphid and was treated with a little nitric acid to insure the oxidation of iron to the ferric condition; or when copper was not determined Solution A was treated directly with nitric acid. The solution, which always contained a large excess of phosphoric acid, was then made

weakly ammoniacal and boiled in order to precipitate the phosphates of the heavy metals and calcium. The filtrate and washings from this precipitate were then treated with a large excess of ammonia and allowed to stand over night to complete the precipitation of magnesium. The filtrate from the magnesium precipitate was reserved for the determination of the alkalies (Solution B), while the combined phosphate precipitates were dissolved in hydrochloric acid to form Solution C, which served for the determination of the heavy metals and the alkaline earths.

*Determination of iron.*—When iron was determined, Solution C, was rendered slightly alkaline with ammonia, and the precipitate thus formed was redissolved by the careful addition of hydrochloric acid. Iron and aluminum phosphates were then precipitated by adding the appropriate quantity of ammonium acetate to the boiling solution. The precipitate thus obtained was filtered off, dissolved in acid, and reprecipitated in the same manner. The second precipitate was filtered off, washed, and dissolved in dilute acid, after which iron was determined by reduction with metallic zinc and titration with decinormal permanganate.

*Determination of alkaline earths.*—Solution C, or, where iron was determined, the combined filtrates from the iron determinations, were freed from interfering metals and from  $P_2O_5$ , and used for the determination of calcium and magnesium as described in the methods adopted by the Association of Official Agricultural Chemists for the determination of manganese, calcium, and magnesium in inorganic plant constituents (1, p. 17), except that the calcium was estimated by titrating the calcium oxalate in sulphuric-acid solution with decinormal potassium permanganate.

*Determination of alkali metals.*—Solution B, which served for the determination of the alkali metals, was brought to a volume of 250 cc. in a volumetric flask. A 100 cc. aliquot of this solution was transferred to a 200 cc. volumetric flask and rendered neutral to methyl red, after which it was treated with 4 cc. of 5 N ammonium acetate, 2 cc. of 5 N acetic acid, and a slight but definite excess of ferric chlorid. The solution was diluted to exactly 200 cc. and filtered through a dry, folded filter from the bulky precipitate of ferric phosphate. From 100 cc. of this filtrate, the excess iron was removed as the basic acetate by boiling and filtering; after which the new filtrate, combined with the washings, was treated with a few drops of dilute sulphuric acid and brought to dryness, the residue being ignited cautiously to expel the ammonium salts. The residue was then dissolved in a small quantity of water, treated with small quantities of ammonium hydroxid and ammonium carbonate, and the solution filtered into a weighed platinum dish. After evaporation the residue of sodium and potassium sulphate was brought to constant weight and weighed. Potassium was determined by the Lindo-Gladding method (1, p. 12) and sodium by difference.

In general, all determinations were made in duplicate and the average of closely agreeing duplicate determinations is reported.

TABLE I.—Composition of livers, expressed in terms of percentages of fresh material

Serial No.	Description of sample	Moisture	Ether extract	Total nitrogen	Purin nitrogen	Total creatinin nitrogen	Glycogen	Dextrose	Total carbohydrate expressed as dextrose	Total phosphorus	Inorganic phosphorus	Organic phosphorus	Copper	Iron	Calcium	Magnesium	Potassium	Sodium
		Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
513	Ox liver	72.28	2.04	3.18	0.110	0.14	0.14	2.24	3.66	0.372	0.125	0.247	0.0016	0.006	0.006	0.021	0.303	0.091
514	do.	70.40	2.10	3.22	0.119	0.14	0.14	2.84	3.66	0.372	0.096	0.276	0.0052	0.005	0.005	0.019	0.289	0.079
515	do.	68.68	2.00	3.17	0.124	0.15	0.15	3.20	3.90	0.345	0.096	0.249	0.0052	0.005	0.005	0.020	0.295	0.075
516	do.	67.56	4.86	3.46	0.121	0.12	0.12	2.92	5.72	0.320	0.088	0.231	0.0050	0.005	0.005	0.019	0.291	0.077
517	do.	68.05	2.37	3.46	0.110	0.15	0.15	2.57	4.02	0.366	0.100	0.266	0.0057	0.006	0.006	0.019	0.274	0.079
518	do.	70.79	2.53	3.30	0.124	0.16	0.16	1.13	4.52	0.376	0.110	0.265	0.0040	0.006	0.006	0.021	0.300	0.080
519	do.	68.57	4.11	3.41	0.113	0.16	0.16	3.35	3.62	0.363	0.096	0.287	0.0049	0.005	0.005	0.020	0.292	0.085
520	do.	70.88	3.83	3.26	0.121	0.17	0.17	2.89	2.70	0.336	0.088	0.248	0.0030	0.005	0.005	0.017	0.254	0.085
521	do.	73.25	2.96	3.30	0.134	0.17	0.17	1.01	1.19	0.373	0.094	0.279	0.0027	0.005	0.005	0.018	0.235	0.111
	Averages for ox livers	70.05	2.98	3.26	0.123	0.14	0.14	2.22	3.53	0.358	0.099	0.259	0.0039	0.0056	0.005	0.019	0.281	0.086
524	Calf liver	70.05	6.15	2.92	0.131	0.14	0.14	1.33	2.04	0.351	0.097	0.254	0.0031	0.0056	0.006	0.020	0.308	0.074
525	do.	68.69	4.62	2.80	0.133	0.18	0.18	2.71	3.94	0.349	0.083	0.266	0.0012	0.005	0.005	0.020	0.330	0.075
526	do.	67.78	7.99	3.18	0.146	0.12	0.12	1.19	1.83	0.361	0.093	0.268	0.0073	0.006	0.006	0.019	0.305	0.081
530	do.	71.53	4.35	3.31	0.155	0.15	0.15	0.50	0.60	0.355	0.102	0.253	0.0048	0.006	0.006	0.020	0.313	0.088
531	do.	70.41	4.71	3.40	0.145	0.15	0.15	0.36	0.378	0.378	0.109	0.289	0.0030	0.0056	0.0056	0.021	0.308	0.091
532	do.	70.50	4.63	3.31	0.145	0.15	0.15	0.32	0.43	0.370	0.108	0.262	0.0076	0.0050	0.0050	0.021	0.304	0.086
	Averages for calf livers	69.83	5.41	3.15	0.142	0.15	0.15	1.04	1.57	0.361	0.099	0.262	0.0045	0.0059	0.0059	0.020	0.311	0.082
534	Hog liver	71.88	5.45	3.32	0.164	0.16	0.16	0.00	0.00	0.382	0.095	0.262	0.0005	0.0077	0.0077	0.022	0.306	0.091
535	do.	73.21	6.41	2.92	0.153	0.18	0.18	0.00	0.00	0.351	0.093	0.266	0.0003	0.005	0.005	0.022	0.289	0.084
536	do.	71.76	3.11	2.91	0.144	0.14	0.14	0.00	0.00	0.352	0.093	0.266	0.0003	0.005	0.005	0.021	0.315	0.083
556	do.	73.71	3.55	3.23	0.186	0.17	0.17	0.28	0.28	0.372	0.088	0.274	0.0004	0.006	0.006	0.021	0.284	0.091
557	do.	72.23	2.90	3.48	0.170	0.17	0.17	0.86	0.86	0.381	0.098	0.283	0.0004	0.0076	0.0076	0.021	0.284	0.091
558	do.	74.25	3.16	3.16	0.165	0.16	0.16	0.39	0.39	0.375	0.094	0.281	0.0004	0.0085	0.0085	0.020	0.286	0.093
	Averages for hog livers	72.84	5.28	3.17	0.164	0.16	0.16	0.25	0.25	0.369	0.097	0.279	0.0004	0.0081	0.0081	0.021	0.296	0.090

TABLE II.—Composition of livers, expressed in terms of percentages of dry, fat-free material

Serial No.	Description of sample	Total nitrogen	Purin nitrogen	Total creatinin nitrogen	Glycogen	Dextrose	Total carbohydrate expressed as dextrose	Total phosphorus	Inorganic phosphorus	Organic phosphorus	Copper	Iron	Calcium	Magnesium	Potassium	Sodium
		Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
513	Ox liver.....	12.38	0.428	.....	0.56	8.72	9.34	1.449	0.488	0.962	0.0631	0.025	0.024	0.081	1.180	0.356
514	do.....	11.71	.....	.....	2.75	10.32	13.30	1.353	0.350	1.003	0.0190	.017	.020	.068	1.032	.286
515	do.....	10.80	0.423	0.051	8.56	10.91	20.16	1.178	0.328	0.850	0.0110	.....	.019	.067	1.005	.256
516	do.....	10.86	0.439	0.043	10.59	9.32	20.77	1.160	0.321	0.839	0.0181	.....	.020	.069	1.056	.278
517	do.....	11.70	0.372	0.050	3.82	5.37	9.48	1.237	0.338	0.899	0.0194	.....	.019	.066	1.027	.266
518	do.....	12.37	0.465	0.061	1.31	6.74	8.15	1.409	0.413	0.996	0.0151	.....	.024	.078	1.122	.290
519	do.....	12.48	0.414	.....	3.27	9.72	13.25	1.331	0.353	0.978	0.0180	.....	.017	.073	1.069	.310
520	do.....	12.89	0.478	.....	2.25	8.26	10.68	1.329	0.347	0.982	0.0117	.....	.020	.069	1.006	.375
521	do.....	13.86	0.563	.....	.70	4.26	5.02	1.569	0.396	1.173	0.0114	.....	.019	.075	.989	.466
	Averages for ox livers.....	12.13	.462	.051	3.76	8.19	12.24	1.335	.370	.965	.0144	.021	.020	.072	1.045	.321
524	Calf liver.....	12.26	.550	.061	2.77	5.57	8.56	1.473	.407	1.066	.0129	.....	.025	.085	1.294	.309
525	do.....	10.48	.498	.068	4.24	10.18	14.75	1.309	.313	.996	.0045	.....	.024	.073	1.236	.282
526	do.....	13.11	.603	.049	2.44	4.91	7.55	1.490	.385	1.105	.0302	.....	.027	.077	1.259	.333
530	do.....	13.71	.622	.....	3.37	2.09	2.50	1.473	.423	1.050	.0201	.....	.025	.085	1.300	.365
531	do.....	13.64	.622	.....	1.46	.79	2.36	1.519	.439	1.080	.0213	.....	.022	.085	1.237	.364
532	do.....	13.30	.584	.....	.40	1.29	1.72	1.490	.435	1.055	.0304	.....	.020	.085	1.222	.347
	Averages for calf livers.....	12.77	.555	.059	1.95	4.15	6.26	1.463	.401	1.051	.0185	.....	.024	.082	1.261	.334
534	Hog liver.....	14.65	.725	.....	0	0	0	1.686	.....	.....	.0021	.....	.034	.097	1.353	.402
535	do.....	14.33	.753	.....	0	0	0	1.722	.....	.....	.0016	.....	.042	.108	1.417	.413
536	do.....	14.46	.714	.....	0	0	0	1.750	.....	.....	.0013	.....	.036	.107	1.567	.463
556	do.....	14.19	.819	.....	0	1.23	1.23	1.637	.432	1.205	.0018	.....	.041	.091	.....	.....
557	do.....	13.97	.683	.....	0	3.47	3.47	1.533	.395	1.138	.0016	.....	.031	.084	1.142	.365
	Averages for hog livers.....	14.32	.744	.....	0	.94	.94	1.665	.413	1.171	.0017	.....	.037	.097	1.369	.410

TABLE III.—Chemical composition of various edible viscera other than liver

Serial No.	Description of sample	Composition of undried material										Compositions of dry, fat-free material								
		Mois- ture	Ether extract	Total nitro- gen	Purin nitro- gen	Glyco- gen	Dex- trose	Total carbo- hy- drates ex- pressed as dex- trose	Inor- ganic phos- phorus	Or- ganic phos- phorus	Ash	Total nitro- gen	Purin nitro- gen	Glyco- gen	Dex- trose	Total carbo- hy- drates ex- pressed as dex- trose	Total phos- phorus	Inor- ganic phos- phorus	Or- ganic phos- phorus	Ash
563	Ox heart.....	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
564	do.....	67.12	15.43	2.74	0.076	0.07	0.186	0.061	0.125	15.70	0.44	0.43	1.066	0.351	0.715	1.111	1.036	0.327	0.709	774
565	do.....	64.12	20.04	2.44	0.072	0.08	0.176	0.053	0.123	15.43	0.45	0.53	1.066	0.351	0.715	1.111	1.036	0.327	0.709	774
571	do.....	70.24	12.00	2.61	0.084	0.08	0.184	0.058	0.126	14.69	0.47	0.43	1.066	0.327	0.709	1.111	1.036	0.327	0.709	774
572	do.....	75.76	4.22	2.96	0.095	0.04	0.32	0.220	0.066	14.77	0.48	0.21	1.066	0.327	0.709	1.111	1.036	0.327	0.709	774
573	do.....	75.92	4.21	2.87	0.087	0.02	0.27	0.213	0.063	14.45	0.44	0.12	1.066	0.327	0.709	1.111	1.036	0.327	0.709	774
	do.....	77.15	2.74	3.01	0.094	0.19	0.45	0.227	0.068	14.98	0.47	0.94	1.128	0.337	0.791	2.24	1.128	0.337	0.791	791
	Averages for ox hearts.....	71.72	9.77	2.77	0.085	0.08	0.36	0.201	0.061	15.00	0.46	0.44	1.085	0.333	0.752	1.79	1.085	0.333	0.752	752
575	10 hog hearts.....	79.29	1.63	2.83	0.082	0.00	0.19	0.209	0.075	14.83	0.43	0.00	1.095	0.391	0.704	1.00	1.095	0.391	0.704	704
576	10 lamb hearts.....	77.35	7.24	1.72	0.051	0.00	0.00	0.346	0.068	11.15	0.34	0.00	2.249	0.443	1.806	0.00	2.249	0.443	1.806	1806
588	10 ox brains.....	77.95	6.82	1.70	0.053	0.00	0.00	0.345	0.051	11.15	0.35	0.00	2.265	0.335	1.930	0.00	2.265	0.335	1.930	1930
593	22 ox brains.....	77.65	7.03	1.71	0.052	0.00	0.00	0.345	0.059	11.15	0.34	0.00	2.257	0.389	1.868	0.00	2.257	0.389	1.868	1868
	Averages for ox brains.....	77.96	7.61	1.62	0.039	0.00	0.00	0.359	0.083	11.43	0.27	0.00	2.488	0.572	1.916	0.00	2.488	0.572	1.916	1916
595	50 hog brains.....	78.77	7.36	1.68	0.038	0.00	0.00	0.325	0.070	12.13	0.27	0.00	2.341	0.506	1.835	0.00	2.341	0.506	1.835	1835
682	do.....	78.36	7.48	1.65	0.038	0.00	0.00	0.342	0.076	11.78	0.27	0.00	2.414	0.539	1.875	0.00	2.414	0.539	1.875	1875
	Averages for hog brains.....	78.26	7.48	1.71	0.046	0.00	0.00	0.333	0.071	11.96	0.32	0.00	2.339	0.501	1.838	0.00	2.339	0.501	1.838	1838
600	70 sheep brains.....	80.12	2.07	2.52	0.083	0.00	0.00	0.230	0.090	14.14	0.46	0.00	1.288	0.505	0.783	0.00	1.288	0.505	0.783	783
604	10 beef kidneys.....	76.25	5.41	2.72	0.095	0.00	0.00	0.264	0.103	14.81	0.52	0.00	1.439	0.562	0.877	0.00	1.439	0.562	0.877	877
606	60 hog kidneys.....	75.92	4.21	2.87	0.087	0.02	0.27	0.213	0.063	14.45	0.44	0.12	1.066	0.327	0.709	1.111	1.036	0.327	0.709	709
612	Ox tongue.....	77.15	2.74	3.01	0.094	0.19	0.45	0.227	0.068	14.98	0.47	0.94	1.128	0.337	0.791	2.24	1.128	0.337	0.791	791
613	do.....	71.72	9.77	2.77	0.085	0.08	0.36	0.201	0.061	15.00	0.46	0.44	1.085	0.333	0.752	1.79	1.085	0.333	0.752	752





## DISCUSSION OF RESULTS

The results obtained from the analyses of the edible viscera are reported in Tables I, II, and III. For purposes of comparison, Table IV, showing the average composition of beef muscle from different parts of the carcass, has been compiled from analyses previously reported in another connection (8).

In general, the data embodied in Tables I, II, and III correspond with what is known regarding the physiological functions of the several organs, and with such analytical data as are to be found in the general textbooks. Since most of the organs are the seat of intense physiological activity, and are correspondingly rich in cells, while some are known to be comparatively rich in phospholipins, high percentages of phosphorus, and more especially of organically combined phosphorus, were to have been expected. The deficiency of carbohydrates in hog liver was unexpected, however, in view of the well-known function of the liver as a storage depot for glycogen; and the reason for this deficiency is not clear.

In so far as the data bear upon the question of nutritive value, they show that the edible viscera are preeminently nitrogenous foods, and as such are most properly compared with meat as a standard. A practical equivalence between lean beef muscle, and the livers of ox, calf, and hog, in this respect, is indicated, while the other organs examined were all inferior to beef muscle in their nitrogen content. With the exception of hearts, tongues, lungs, and stomachs, on the other hand, the edible viscera examined were found to be richer in phosphorus than is beef muscle.

The chemical composition of a food, however, can no longer be regarded as constituting in and of itself a sufficient index of its nutritive value; so that further discussion of the nutritive value of the edible viscera will be postponed until the studies now in progress on the biological value of their proteins and on their value as sources of the several vitamins shall have been completed.

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# FUMIGATION OF BEAN WEEVILS, *BRUCHUS OBTECTUS* SAY AND *B. QUADRIMACULATUS* FAB.<sup>1</sup>

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## INTRODUCTION

In the course of the bean weevil investigations carried on in California during the last four years, the writer has had an opportunity to make observations on the methods of fumigation and their effectiveness when applied to bean weevils.<sup>2</sup>

He has carried on fumigation experiments using carbon disulphide, hydrocyanic-acid gas obtained from liquefied hydrocyanic acid, and hydrocyanic-acid gas generated by the pot method, the amounts of beans fumigated varying from only a small quantity to 300 centals, or 30,000 pounds, at a time.

Liquefied hydrocyanic-acid gas, as used in these experiments, was the commercial hydrocyanic-acid gas. This material contains not less than 96 per cent actual hydrocyanic acid, and is free from alkalies, ammonia, chlorine, hydrochloric acid, and nitric acid. For economy of space, this material is designated as Liq. HCN in the tables of results.

The material used in the pots for the generation of hydrocyanic-acid gas was commercial sodium cyanide labeled 98-99 per cent pure and commercial sulphuric acid 66° B.

The beans, California Pink and Red Mexican, and the cowpeas, Large Blackeye, Red Ripper, and Black-Spotted or Holstein, used in these experiments, are thought to be typical examples of beans and cowpeas, and it is believed that a fumigant will penetrate other varieties in approximately the time required to penetrate these. The bean weevils, *Bruchus obtectus* Say, were contained within and among the California Pink, Red Mexican, and Large Blackeyed cowpeas, while the four-spotted cowpea weevils, *B. quadrimaculatus* Fab., were within and among the cowpeas only.

In these experiments carbon disulphide was used in quantities varying from 1.5 to 31 pounds per 1,000 cubic feet, at temperatures of from 52° to 82° F., and with exposures of from 1 to 90 hours, as well as in a vacuum of 29 inches. Liquefied hydrocyanic acid was used in amounts from 1.3 to 3.5 pounds<sup>3</sup> per 1,000 cubic feet at temperatures of from 58° to 92° F. for lengths of time varying from 24 to 90 hours. In generating hydrocyanic-acid gas by the pot method, from 1.8 to 3.4 pounds of sodium cyanide was used per 1,000 cubic feet at 67° to 84° F. for from 48 to 90 hours.

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<sup>1</sup> Received for publication April 19, 1924.

<sup>2</sup> The writer began the bean weevil investigation in 1919 under the direction of Dr. E. A. Back, entomologist in charge of stored-product insect investigations of the Bureau of Entomology, United States Department of Agriculture. In the fumigation work he has been assisted at various times by E. Nelson, B. Smit, and A. H. Amis, all of whom have rendered assistance in various ways.

He wishes to thank especially D. B. Mackie, of the State department of agriculture of California, Sacramento, Calif., C. L. Main, of the Puente Milling & Warehouse Co., Puente, Calif., and E. R. Hulbirt, of the Owl Fumigation Corporation, Azusa, Calif., for their valuable cooperation, and for the services they so courteously rendered.

<sup>3</sup> In citrus fumigation 18 cc. of liquefied hydrocyanic acid is considered to be the equivalent of 1 ounce of sodium cyanide, but 20 cc. is more nearly the equivalent at 60° F. At 60° F., 642 cc. of liquefied hydrocyanic acid weighs 1 pound; at 70° F., 650 cc. of liquefied hydrocyanic acid weighs 1 pound; at 80° F., 659 cc. of liquefied hydrocyanic acid weighs 1 pound.

The investigations have shown that fumigation of beans as it is done frequently in warehouses, outside of warehouses under canyas covers, and in loaded freight cars does not give satisfactory results. Even in fumigators specially constructed, within or adjoining the warehouses, the kill is not always complete, and the statement is commonly made that fumigation does not kill the eggs or immature stages.

In cases where carbon disulphide was used, the unsatisfactory results are not to be wondered at when one considers the statements of scientific workers along this line. In 1895 Osborn and Mally (4)<sup>4</sup> said: "For carbon disulphide to be effective there must be two or three applications about three or four weeks apart."

Pettit (6), in 1906, said: "Fumigation of the seed, during a warm spell, using carbon disulphide in the regular way . . . should kill all the active beetles, and a second treatment, after a period of warm weather, should complete the work, if indeed the first does not suffice."

Krall, in 1914 (2), questioned the possibility of killing all immature forms with one application. Metcalf (3), in 1916, when reporting work done in 1912 with *Bruchus chinensis* L. and *B. quadrimaculatus*, says that he fumigated them with carbon disulphide, "using 4 pounds to 1,000 cubic feet of air space in a very tight bin at 74° for 72 hours. Contrary to our expectation, however, the cowpea weevils continued to breed in undiminished numbers, although a great many adults were killed at the time the fumigation was done."

Wade (7), in 1919, says: "One of the first difficulties encountered in our tests was to kill all stages of the weevil in cowpeas by fumigating with carbon disulphide for the usual length of time. It was determined before proceeding far that all of the immature stages were not overcome by the fumigant, especially where several bushels were treated."

In view of the foregoing the writer undertook to determine which stage of the weevils, *B. obtectus* and *B. quadrimaculatus*, was the most resistant and which stage was least resistant to fumigation with carbon disulphide or hydrocyanic-acid gas.

#### EFFECT OF FUMIGATION UPON THE EMERGED ADULT

Table I shows that 2 pounds of carbon disulphide per 1,000 cubic feet at a temperature of 53° to 61° F. killed all emerged adult bean weevils, *Bruchus obtectus*, with an exposure of 24 hours. Three pounds of carbon disulphide per 1,000 cubic feet at 62° to 68° F. killed all emerged adults in 21 hours. One and one-half pounds of carbon disulphide per 1,000 cubic feet at 52° to 68° F. killed all adults in 48 hours.

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<sup>4</sup> Reference is made by number (*italic*) to "Literature cited," p. 356.

TABLE I.—The effect of fumigation on adult weevils, *Bruchus obtectus*

Number of weevils	Date. fumigated	Material used	Dosage per 1,000 cubic feet	Exposure	Number alive	Number dead	Temperature		Humidity	
							Maximum	Minimum	Maximum	Minimum
			Lbs.	Hrs.			° F.	° F.		
100	Apr. 19	CS <sub>2</sub>	2	24	0	100	61	53	70	66
100	do.	do.	2	24	0	100	61	53	70	66
50	do.	do.	2	24	0	50	61	53	70	66
100	(a)				100	0	76	49	68	41
100	Apr. 20	CS <sub>2</sub>	3	24	0	100	58	53	70	66
100	do.	do.	3	24	0	100	58	53	70	66
50	do.	do.	3	24	0	50	58	53	70	66
100	(a)				100	0	75	49	75	45
100	Apr. 21	CS <sub>2</sub>	1.5	48	0	100	68	60	64	59
100	(a)				100	0	85	50	70	29
100	Apr. 23	CS <sub>2</sub>	1.5	48	0	100	60	52	68	59
100	(a)				100	0	69	41	68	19
100	May 13	CS <sub>2</sub>	3	21	0	100	68	62	73	71
100	(a)				100	0	81	60	82	53
50	May 17	Liq. HCN	1.8	24	0	50	70	62	70	45
50	do.	do.	1.8	24	0	50	70	62	70	45
50	(a)				50	0	85	51	92	44
50	June 8	CS <sub>2</sub>	6	21	0	50	68	64	70	66
50	(a)				50	0	87	50	83	50
50	June 11	Liq. HCN	3.5	48	0	50	75	62	80	58
50	do.	do.	3.5	48	0	50	75	62	80	58
50	do.	do.	3.5	48	0	50	75	62	80	58
50	(a)				50	0	83	64	82	54
50	(a)				50	0	83	64	82	54
50	(a)				50	0	83	64	82	54

a Control.

b All fumigation by the use of liquified hydrocyanic acid was done in cooperation with E. R. Hulbirt of the Owl Fumigation Corporation of Azusa, Calif.

Table II shows that all emerged adult four-spotted cowpea weevils, *Bruchus quadrimaculatus*, were killed by an exposure of 24 hours to 1.8 pounds of liquefied hydrocyanic acid at 62° to 70° F., and that 1.3 pounds was equally effective when the exposure was extended to 90 hours at a temperature ranging from 58° to 91° F. In a 29-inch vacuum where carbon disulphide was used at the rate of 31 pounds per 1,000 cubic feet at a temperature of 82° F. all emerged adults were killed in one hour.

TABLE II.—The effect of fumigation on adult weevils, *Bruchus quadrimaculatus*

Number of weevils	Date. fumigated	Material used	Dosage per 1,000 cubic feet	Exposure	Number alive	Number dead	Temperature		Humidity	
							Maximum	Minimum	Maximum	Minimum
			Lbs.	Hrs.			° F.	° F.		
50	May 17	Liq. HCN	1.8	24	0	50	70	62	70	45
50	(a)				50	0	85	51	92	44
50	Aug. 16	Liq. HCN	2	48	0	50	92	64	79	46
50	do.	do.	2	48	0	50	92	64	79	46
50	(a)				48	2	92	64	79	46
50	(a)				47	3	92	64	79	46
50	Aug. 17	CS <sub>2</sub> and 29-inch vacuum. <sup>b</sup>	31	1	0	50	82	82	-----	-----
50	do.	do.	31	1	0	50	82	82	-----	-----
50	(a)				50	0	92	64	79	46
50	(a)				50	0	92	64	79	46
50	Aug. 18	Liq. HCN	1.3	90	0	50	91	58	79	43
50	do.	do.	1.3	90	0	50	91	58	79	43
50	(a)				45	5	91	58	79	43
50	(a)				46	4	91	58	79	43
50	Aug. 23	HCN, pot method <sup>c</sup>	<sup>d</sup> 3.4	48	0	50	82	69	71	51
50	do.	do.	<sup>d</sup> 3.4	48	0	50	82	69	71	51
50	do.	do.	<sup>d</sup> 3.4	48	0	50	82	69	71	51
50	(a)				50	0	82	69	71	51
50	Aug. 25	HCN, pot method <sup>c</sup>	1.8	90	0	<sup>e</sup> 50	84	67	66	51
50	(a)				<sup>e</sup> 50	0	84	67	66	51

a Control.

b In the experiments with vacuum fumigation the material was prepared by the writer and fumigated by Mr. D. B. Mackie, of the California State Department of Agriculture, after which tabulations were made by the writer.

c For economy of space in the tables, "HCN, pot method" is used to designate hydrocyanic-acid gas generated by the pot method.

d Dosage of sodium cyanide employed to generate the gas.

e These weevils emerged on Aug. 24.

EFFECT OF FUMIGATION UPON THE EGG

Although Table III shows that 2 pounds of carbon disulphide per 1,000 cubic feet at 53° to 61° F. for 24 hours did not kill all the eggs of *Bruchus obtectus*, it shows that 3 pounds at 53° to 58° F. prevented the hatching of any eggs, and 1½ pounds at temperature only slightly higher killed all eggs in 48 hours. One and eight-tenths pounds of liquefied hydrocyanic acid per 1,000 cubic feet prevented the hatching of any eggs when exposed 24 hours at 62° to 70° F.

TABLE III.—Effect of different kinds of fumigation for varying lengths of time on eggs of *Bruchus obtectus*

Num-ber of eggs	Date de-posited	Date treated	Material used	Dos-age per 1,000 cubic feet	Ex-po-sure	Num-ber hatch-ed	Num-ber failed to hatch	Temperature		Humidity	
								Maxi-mum	Mini-mum	Maxi-mum	Mini-mum
				Lbs.	Hrs.			° F.	° F.		
100	Apr. 18	Apr. 19	CS <sub>2</sub> -----	2	24	10	90	61	53	70	66
50	do-----	do-----	do-----	2	24	0	50	61	53	70	66
100	do-----	(a)	-----			89	11	76	49	68	41
50	do-----	(a)	-----			49	1	76	49	68	41
100	Apr. 19	Apr. 20	CS <sub>2</sub> -----	3	24	0	100	58	53	69	66
100	do-----	(a)	-----			94	6	75	49	75	45
100	Apr. 20	Apr. 21	CS <sub>2</sub> -----	1.5	48	0	100	68	60	64	59
50	do-----	do-----	do-----	1.5	48	0	50	68	60	64	59
50	do-----	(a)	-----			46	4	85	50	70	29
50	Apr. 22	Apr. 23	CS <sub>2</sub> -----	1.5	48	0	50	60	52	68	59
50	do-----	(a)	-----			41	9	69	41	68	19
100	May 12	May 13	CS <sub>2</sub> -----	3	21	0	100	68	62	73	71
100	do-----	(a)	-----			83	17	81	60	82	53
50	May 16	May 17	Liq. HCN-----	1.8	24	0	50	70	62	70	45
50	do-----	do-----	do-----	1.8	24	0	50	70	62	70	45
50	do-----	(a)	-----			47	3	85	51	92	44
50	June 7	June 8	CS <sub>2</sub> -----	6	21	0	50	68	64	70	66
50	do-----	do-----	do-----	6	21	0	50	68	64	70	66
50	do-----	(a)	-----			46	4	87	50	83	50
50	June 10	June 11	Liq. HCN-----	3.5	48	0	50	75	62	80	58
50	do-----	do-----	do-----	3.5	48	0	50	75	62	80	58
50	do-----	do-----	do-----	3.5	48	0	50	75	62	80	58
50	do-----	do-----	do-----	3.5	48	0	50	75	62	80	58
50	do-----	(a)	-----			44	6	83	64	82	54
50	do-----	(a)	-----			46	4	83	64	82	54
50	do-----	(a)	-----			44	6	83	64	82	54

a Control.

The most striking thing shown in Table IV is that at a temperature of 62° to 70° F. the viability of all the eggs of *Bruchus quadrimaculatus* was not destroyed by the use of 1.8 pounds of liquefied hydrocyanic acid per 1,000 cubic feet for 24 hours, although the same dosage (see Table III) killed all eggs of *Bruchus obtectus*.

The eggs recorded in Tables III and IV were so arranged that there were eggs which had been deposited less than 24 hours, other eggs in every stage of development, and some just beginning to hatch.

TABLE IV.—Effect of different kinds of fumigation for varying lengths of time on eggs of *Bruchus quadrimaculatus*

Number of eggs	Date deposited	Date treated	Material used	Dosage per 1,000 cubic feet	Exposure	Number hatched	Number failed to hatch	Temperature		Humidity	
								Maximum	Minimum	Maximum	Minimum
				Lbs.	Hrs.			°F.	°F.	°F.	°F.
50	May 16	May 17	Liq. HCN	1.8	24	0	50	70	62	70	45
50	do	do	do	1.8	24	0	50	70	62	70	45
50	do	(a)	do			42	8	85	51	92	44
39	do	May 17	do	1.8	24	1	38	70	62	70	45
38	do	(a)	do			28	10	85	51	92	44
50	June 7	June 8	CS <sub>2</sub>	6	21	0	50	68	64	70	66
50	do	do	do	6	21	0	50	68	64	70	66
50	do	(a)	do			43	7	87	50	83	50
50	do	(a)	do			49	1	87	50	83	50
50	June 10	June 11	Liq. HCN	3.5	48	0	50	75	62	80	58
50	do	do	do	3.5	48	0	50	75	62	80	58
50	do	do	do	3.5	48	0	50	75	62	80	58
50	do	do	do	3.5	48	0	50	75	62	80	58
50	do	(a)	do			47	3	83	64	82	54
50	do	(a)	do			49	1	83	64	82	54
50	do	(a)	do			43	7	83	64	82	54
50	Aug. 15	Aug. 16	Liq. HCN	2	48	0	50	92	64	79	46
50	do	do	do	2	48	0	50	92	64	79	46
50	do	(a)	do			44	6	92	64	79	46
50	do	(a)	do			36	14	92	64	79	46
50	do	Aug. 17	CS <sub>2</sub> and 29 inch-vacuum.	31	1	0	50	82	82		
50	do	do	do	31	1	0	50	82	82		
50	do	(a)	do			44	6	92	64	79	46
50	do	(a)	do			36	14	92	64	79	46
50	Aug. 17	Aug. 18	Liq. HCN	1.3	90	0	50	91	58	79	43
50	do	do	do	1.3	90	0	50	91	58	79	43
50	do	(a)	do			<sup>b</sup> 37	13	91	58	79	43
50	do	(a)	do			<sup>b</sup> 35	15	91	58	79	43
50	Aug. 22	Aug. 23	HCN, pot method.	<sup>c</sup> 3.4	48	0	50	82	69	71	51
50	do	do	do	<sup>c</sup> 3.4	48	0	50	82	69	71	51
50	do	(a)	do			<sup>b</sup> 37	13	82	69	71	51
50	do	(a)	do			<sup>b</sup> 35	15	82	69	71	51
50	Aug. 24	Aug. 25	HCN, pot method.	<sup>c</sup> 1.8	90	0	50	84	67	66	51
50	do	do	do	<sup>c</sup> 1.8	90	0	50	84	67	66	51
50	do	(a)	do			34	<sup>d</sup> 11	84	67	66	51
50	do	(a)	do			28	<sup>d</sup> 22	84	67	66	51

<sup>a</sup> Control.<sup>b</sup> Figures seem to indicate that the controls were identical on August 17 and August 22, which is not the case.<sup>c</sup> Dosage of sodium cyanide employed to generate the gas.<sup>d</sup> Mites, *Pediculoides ventricosus* Newp., probably account for the low hatch and in one of the experiment the five eggs unaccounted for.

## EFFECT OF FUMIGATION UPON FORMS WITHIN THE SEEDS

The data in Table V indicate that 2 pounds of carbon disulphide per 1,000 cubic feet at 53° to 61° F. for 24 hours was not effective in killing the unemerged adults, pupæ, full-grown larvæ, or half-grown larvæ, of *Bruchus obtectus*, but was effective in killing the young larvæ. The same dosage gave a complete kill when adults free of seeds were treated. (See Table I.) Three pounds of carbon disulphide at 53° to 58° F. for 24 hours made a complete kill, as did 1½ pounds for 48 hours at 60° to 68° F. One and eight-tenths pounds of liquefied hydrocyanic acid per 1,000 cubic feet at 62° to 70° F. for 24 hours did not kill all the unemerged adults and pupæ, but it killed the larvæ. The same dosage killed all eggs of *B. obtectus* and emerged adults of both species of weevils. (See Tables I to IV.)

TABLE V.—Percentage of weevils, *Bruchus obtectus*, that were dead within the beans after being fumigated with different amounts of carbon disulphide or hydrocyanic-acid gas for varying lengths of time <sup>a</sup>

Amount fumigated	Material used	Dosage per 1,000 cubic feet	Exposure	Un-emerged adults		Pupæ		Larvæ						Temperature		Humidity	
				Alive	Dead	Alive	Dead	Full-grown		Half-grown		Young		Maximum	Minimum	Maximum	Minimum
								Alive	Dead	Alive	Dead	Alive	Dead				
Centals.		Lbs.	Hrs.											°F.	°F.		
13	CS <sub>2</sub> -----	2	24	58	42	54	46	6	94	6	94	0	100	61	53	70	66
	Control-----			84	16	81	19	60	40	50	50	0	0	76	49	68	41
13	CS <sub>2</sub> -----	2	24	37	63	38	62	25	75	0	0	0	0	61	53	70	63
	Control-----			82	18	98	2	67	33	0	0	0	0	76	49	68	41
9	CS <sub>2</sub> -----	3	24	0	100	0	100	0	100	0	0	0	0	58	53	69	66
	Control-----			75	25	94	6	53	47	0	0	0	0	75	49	75	45
9	CS <sub>2</sub> -----	3	24	0	100	0	100	0	100	0	0	0	0	58	53	69	66
	Control-----			64	36	88	12	100	0	0	0	0	0	75	49	75	45
9	CS <sub>2</sub> -----	1.5	48	0	100	0	100	0	100	0	0	0	0	68	60	64	59
	do-----	1.5	48	0	100	0	100	0	100	0	0	0	0	68	60	64	59
	Control-----			70	30	61	39	79	21	0	0	0	0	85	50	70	29
9	CS <sub>2</sub> -----	1.5	48	0	100	0	100	0	100	0	0	0	0	60	52	68	59
	Control-----			85	15	90	10	0	0	0	0	0	0	69	41	68	19
9	CS <sub>2</sub> -----	1.5	48	18	82	13	87	11	89	0	0	0	0	60	52	68	59
	Control-----			82	18	92	8	100	0	0	0	0	0	69	41	68	19
5	CS <sub>2</sub> -----	3	21	5	95	9	91	35	65	0	0	0	0	68	62	73	71
	Control-----			55	45	59	41	5	95	0	0	0	0	81	60	82	53
210	CS <sub>2</sub> -----	6	21	2	98	26	74	40	60	0	0	0	0	68	64	70	66
	Control-----			97	3	86	14	71	29	0	0	0	0	87	50	83	50
210	CS <sub>2</sub> -----	6	21	20	80	47	53	0	0	0	0	0	0	68	64	70	66
	Control-----			98	2	87	13	67	33	0	0	0	0	87	50	83	50
210	CS <sub>2</sub> -----	6	21	5	95	35	65	10	90	0	0	0	0	68	64	70	66
	do-----	6	21	6	94	60	40	71	29	0	0	0	0	68	64	70	66
	Control-----			90	10	97	3	62	38	0	0	0	0	87	50	83	50
	do-----			96	4	90	10	75	25	0	0	0	0	87	50	83	50
300	Liq. HCN-----	1.8	24	49	51	68	32	0	100	0	0	0	0	70	62	70	45
300	do-----	1.8	24	38	62	56	44	0	0	0	0	0	0	70	62	70	45
300	do-----	1.8	24	52	48	20	80	0	100	0	0	0	0	70	62	70	45
	Control-----			92	8	84	16	88	12	0	0	0	0	85	51	92	44
150	Liq. HCN-----	3.5	48	0	100	0	100	0	100	0	0	0	0	75	62	80	58
	Control-----			94	6	94	6	83	17	0	0	0	0	83	64	82	54
150	Liq. HCN-----	3.5	48	0	100	0	100	0	100	0	0	0	0	75	62	80	50
	Control-----			90	10	82	18	75	25	0	0	0	0	83	64	82	54
150	Liq. HCN-----	3.5	48	0	100	0	100	0	100	0	0	0	0	75	62	80	50
	Control-----			85	15	83	17	50	50	0	0	0	0	83	64	82	54

<sup>a</sup> One hundred weevily beans were placed in each of a number of cotton-stoppered vials and the vials placed in bags of beans in different parts of the fumigator. A like number of weevily beans were removed to the laboratory for controls.

<sup>b</sup> Became heavily infested with mites, *Pediculoides ventricosus*, before they could be dissected.

<sup>c</sup> Six pounds of CS<sub>2</sub> was placed in three shallow pans. In 1.2 hours it was necessary to remove 31 bags; then 1½ pounds of CS<sub>2</sub> was added, and the fumigator was closed after being open less than 5 minutes. Results should be discarded.

Table VI shows that all stages of *Bruchus quadrimaculatus* contained within the black-spotted cowpeas were killed by fumigation with 31 pounds of carbon disulphide per 1,000 cubic feet in a 29-inch vacuum for 1 hour at 82° F., with 2 pounds of liquefied hydrocyanic acid per 1,000 cubic feet for 48 hours at 64° to 92° F., and with 1.3 pounds of liquefied hydrocyanic acid at 58° to 91° F. for 90 hours. The use of 3.4 pounds of sodium cyanide in fumigation with hydrocyanic-acid gas by the pot method at 69° to 82° F. was also 100 per cent effective.



TABLE VI.—Effect of fumigation of weevils, *Bruchus quadrimaculatus*, within black-spotted cowpeas

Material used	Dosage per 1,000 cubic feet	Exposure	Killed	Eggs	Larvæ				Pupæ	Adults		Temperature		Humidity	
					Young	One-fourth grown	One-half grown	Full-grown		Unemerged	Emerged before fumigation	Maximum	Minimum	Maximum	Minimum
CS <sub>2</sub> and 29-inch vacuum.....	Lbs. 31	Hrs. 1	P. ct. 100	172	51	12	6	15	25	24	8	° F. 82	82	-----	-----
Do.....	31	1	100	154	23	8	10	13	36	21	11	82	82	-----	-----
Liq. HCN.....	2	48	100	157	34	20	6	20	27	14	7	92	64	79	46
Do.....	2	48	100	210	28	17	5	26	25	17	9	92	64	79	46
Control.....					28	23	18	17	13	27	51	92	64	79	46
Do.....					171	64	15	18	28	26	47	92	64	79	46
Liq. HCN.....	1.3	90	100	69	39	9	7	17	24	6	4	91	58	79	43
Control.....					113	78	48	43	23	21	70	91	58	79	43
HCN, pot method.....	a 3.4	48	100	-----	25	21	18	17	31	18	-----	82	69	71	51

a Dosage of sodium cyanide employed to generate the gas.

The weevils here listed were in all stages from fresh-laid eggs to weevils just beginning to emerge at the time they were prepared for fumigation. The vacuum fumigation was done at Sacramento and the other at Puente, Calif. The fumigated cowpeas and controls were prepared the same day, but those going to Sacramento were in transit one day longer than the others and consequently more weevils emerged from them before fumigation. The eggs on the fumigated cowpeas were counted at the time they were dissected, but the eggs on the control were not counted because several weevils had emerged from them, thereby removing some of the eggs, and these weevils had also laid numerous eggs on the cowpeas before they were dissected.

On August 28, or 13 days after the first fumigation, the weevils within the controls were all killed, because they became infested with mites, *Pediculoides ventricosus*. Although all the weevils were dead at the time they were dissected from the cowpeas, the tabulation shows that a marked development of the weevils took place within the controls during the 13 days after the others had been killed by fumigation.

In Tables VII and VIII the weevils contained in the first four and the first ten controls, respectively, were killed because of mites when they had developed 13 days after the others were fumigated, while in the other controls the weevils were killed after 16 days' development. In these, as in Table VI, all weevils were dead when they were dissected from the cowpeas. The marked development noted in the controls indicates that all stages had been killed immediately in the fumigated cowpeas.

The work of Paddock and Reinhard (5) indicates that they killed all eggs, larvæ, and pupæ of *Bruchus quadrimaculatus* when they used 4 pounds of carbon disulphide per 1,000 cubic feet for only 24 hours.



TABLE VII.—Effect of fumigation of weevils, *Bruchus quadrimaculatus*, within blackeyed cowpeas

Material used	Dosage per 1,000 cubic feet	Exposure	Killed	Eggs	Larvæ				Pupæ	Adults		Tempera- ture		Humid- ity	
					Young	One-fourth grown	One-half grown	Full-grown		Unemerged	Emerged before fu- migation	Maximum	Minimum	Maximum	Minimum
CS <sub>2</sub> and 29-inch vacuum.....	Lbs. 31	Hrs. 1	P. ct. 100	237	25	3	3	5	46	31	16	82	82	---	---
Do.....	31	1	100	580	20	10	2	7	30	74	32	82	82	---	---
Liq. HCN.....	2	48	100	104	23	2	4	9	54	39	17	92	64	79	46
Do.....	2	48	100	76	17	5	1	3	50	42	17	92	64	79	46
Control.....				81	14	2	2	3	9	8	113	92	64	79	46
Do.....				171	22	21	22	20	21	43	105	92	64	79	46
Liq. HCN.....	1.3	90	100	69	20	3	0	3	44	62	7	91	58	79	43
Do.....	1.3	90	100	40	15	5	2	12	35	57	2	91	58	79	43
Control.....				65	9	2	1	2	18	21	110	91	58	79	43
Do.....				64	7	10	7	12	8	8	99.	91	58	79	43
HCN, pot method.....	*3.4	48	100	---	124	53	51	91	108	128	---	82	69	71	51
Control.....				---	13	12	15	14	24	2,269	---	82	69	71	51
HCN, pot method.....	*1.8	90	100	78	46	5	1	3	21	51	7	84	67	66	61
Do.....	*1.8	90	100	68	25	15	10	1	10	49	14	84	67	66	61

\* Dosage of sodium cyanide used to generate the gas.

TABLE VIII.—Effect of fumigation of weevils, *Bruchus Quadrimaculatus*, within Red Ripper cowpeas

Material used	Dosage per 1,000 cubic feet	Exposure	Killed	Eggs	Larvæ				Pupæ	Adults		Tempera- ture		Humid- ity	
					Young	One-fourth grown	One-half grown	Full-grown		Unemerged	Emerged before fu- migation	Maximum	Minimum	Maximum	Minimum
CS <sub>2</sub> and 29-inch vacuum.....	Lbs. 31	Hrs. 1	P. ct. 100	70	108	57	54	144	31	---	---	° F. 82	° F. 82	---	---
Do.....	31	1	100	63	103	54	53	138	60	---	---	82	82	---	---
Do.....	31	1	100	72	255	171	88	38	---	---	---	82	82	---	---
Do.....	31	1	100	72	163	251	75	33	1	---	---	82	82	---	---
Liq. HCN.....	2	48	100	169	158	72	52	60	1	---	---	92	64	79	46
Do.....	2	48	100	146	160	57	44	65	1	---	---	92	64	79	46
Do.....	2	48	100	71	508	92	18	6	---	---	---	92	64	79	46
Do.....	2	48	100	153	558	25	9	2	---	---	---	92	64	79	46
Control.....				---	23	73	18	18	30	66	191	92	64	79	46
Do.....				---	5	12	13	25	66	144	55	92	64	79	46
Do.....				---	37	10	5	15	25	121	160	92	64	79	46
Do.....				---	3	6	6	16	45	93	251	92	64	79	46
Liq. HCN.....	1.3	90	100	55	136	56	66	108	23	---	---	91	58	79	43
Do.....	1.3	90	100	35	100	50	71	90	29	---	---	91	58	79	43
Do.....	1.3	90	100	75	218	222	72	42	1	---	---	91	58	79	43
Do.....	1.3	90	100	54	163	257	80	53	---	---	---	91	58	79	43
Do.....	1.3	90	100	50	100	37	40	134	46	---	---	91	58	79	43
Do.....	1.3	90	100	74	256	127	78	56	8	---	---	91	58	79	43
Control.....				---	22	24	12	18	29	48	179	91	58	79	43
Do.....				---	12	14	6	12	26	53	168	91	58	79	43
Do.....				---	5	---	8	25	24	121	163	91	58	79	43
Do.....				---	3	28	6	10	34	114	196	91	58	79	43
Do.....				---	10	32	30	56	110	145	91	58	79	43	---
Do.....				---	6	2	3	14	25	70	203	91	58	79	43
HCN, pot method.....	*3.4	48	100	85	146	79	93	68	40	14	3	82	69	71	51
Do.....	*3.4	48	100	57	124	90	63	69	89	9	---	82	69	71	51
Do.....	*3.4	48	100	64	237	109	138	113	11	---	---	82	69	71	51
Do.....	*3.4	48	100	89	152	147	115	141	130	---	---	82	69	71	51
Control.....				---	42	41	43	47	43	72	46	82	69	71	51
Do.....				---	88	78	81	125	49	71	69	82	69	71	51
Do.....				---	19	17	41	29	39	84	100	82	69	71	51
Do.....				---	13	29	24	37	45	70	160	82	69	71	51
HCN, pot method.....	*1.8	90	100	79	137	92	53	159	133	1	---	84	67	66	51
Do.....	*1.8	90	100	70	91	62	30	106	142	19	---	84	67	66	51
Control.....				---	20	34	29	27	117	89	102	84	67	66	51
Do.....				---	12	28	16	40	60	60	131	84	67	66	51

\* Dosage of sodium cyanide employed to generate the gas.

The writer's observations, together with the foregoing experiments, seem to indicate that in the commercial fumigation of beans there are four outstanding causes for the unsatisfactory results obtained. The first is the use of fumigation rooms which are far from being gas-tight, another is the use of a poor grade of carbon disulphide, and the third is carrying on the fumigation at too low temperatures. Where none of these can be given as a cause for unsatisfactory results, a fourth may enter. This is too short an exposure. Reference to the foregoing tables will indicate that a small amount of hydrocyanic-acid gas or of carbon disulphide will do the work as efficiently in from 48 to 90 hours as twice the amount of the same fumigant will in 24 hours.

With reference to carbon disulphide as a fumigant, Hinds (1) says:

"As a matter of fact, most, if not all, of the killing will have occurred during the first 6 hours of exposure, and the building may be ventilated after that time, as a minimum, has elapsed, though it is better to wait 12 hours or longer."

Undoubtedly this is the case when large amounts of carbon disulphide and only small quantities of beans are used, but the writer is convinced that where large quantities of beans are to be fumigated, or where relatively small amounts of the fumigant are used, a period much longer than six hours is required to make a complete kill. The writer finds that if the gas is not strong at the end of a 24-hour exposure, the room is so open that all weevils will not be killed if a large quantity of beans has been fumigated. In such a room a large quantity of the fumigant should be used, and only comparatively small quantities of beans should be fumigated.

Where hydrocyanic-acid gas is used, the room must be sufficiently tight to allow the gas to penetrate the beans before it diffuses out of the room. As this gas is not heavier than air, it requires a much longer time than does carbon disulphide to penetrate the beans thoroughly; therefore, a minimum of 48 hours should be allowed when using it.

A very satisfactory bean fumigator can be constructed at a moderate price, the cost being determined by the size and location of the fumigator. It should have double walls, floor, and ceiling, made of tongue-and-groove, 1-inch lumber lined with tarred paper, having all joints sealed. All openings should be closed with specially constructed doors of the icehouse type or other type which can be sealed air-tight.

A more expensive fumigator can be constructed of concrete or brick, having all joints properly sealed.

### CONCLUSIONS

In commercial work there appears to be an unnecessary fear against the use of hydrocyanic-acid gas, because of its poisonous nature, but with the care that should be exercised in the use of carbon disulphide, hydrocyanic-acid gas can be used with safety.

In commercial work, as well as in scientific work, opinions differ as to the efficiency of carbon disulphide as a fumigant against bean weevils. While this appears to be due principally to three factors, in some instances a fourth factor may enter. These factors are:

1. The use of a room or fumigatorium which is far from being gas-tight.
2. The use of an inferior grade of carbon disulphide.
3. Carrying on the work of fumigation at too low temperatures.
4. (Which may enter when large quantities of beans are being fumigated and when unsatisfactory results can be charged to neither of the first three factors.) Too short an exposure. No. 1, above, frequently resolves itself into too short an exposure.

When using a good grade of either carbon disulphide or hydrocyanic-acid gas in a gas-tight fumigator, at a sufficiently high temperature, and with a sufficiently long exposure, it is entirely possible with one fumigation to kill all the weevils, in all stages, either among or within beans or cowpeas. This, however, will of course not prevent a reinfestation if weevils have access to the fumigated seeds.

Small quantities of beans may be thoroughly fumigated with a minimum of 3 pounds of carbon disulphide per 1,000 cubic feet at a temperature of 58° F. or higher for a period of 24 hours or with 1½ pounds for 48 hours; with two pounds of liquefied hydrocyanic acid for 48 hours at 70° F. or higher or with 1.3 pounds for 90 hours; with hydrocyanic-acid gas generated from 3.4 pounds of sodium cyanide by the pot method at 70° F. for 48 hours or from 1.8 pounds of sodium cyanide for 90 hours.

Large quantities of beans require a longer exposure or a greater quantity of the fumigant, the latter being preferable. When beans are fumigated with hydrocyanic-acid gas they should be given an exposure of at least 48 hours.

When either carbon disulphid or hydrocyanic-acid gas is used as a fumigant, the weevil *Bruchus obtectus* is most easily killed in the young larval stage just after hatching and just before entrance into the bean. Emerged adults, eggs, and young larvæ just after having entered the bean succumb to fumigation in the order named, while it appears that unemerged adults and pupæ are hardest to kill. It appears, however, that the depth to which the weevil is buried in the bean is important as affecting its ability to withstand fumigation.

Stages of *Bruchus quadrimaculatus* are most easily killed in the following order: Emerged adults, eggs, larvæ that have not entirely left the chorion, and young larvæ just inside the seed; the other stages appear to be effected almost equally. The larvæ that are deepest in the cowpeas survive longer than other larvæ. This accounts for the fact that the prepupæ appear to be a little more resistant than the other stages.

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# LEATHER ROT OF STRAWBERRIES<sup>1</sup>

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One of the most destructive diseases of strawberries (*Fragaria* sp.) in the southern Mississippi Valley is a rot of the fruit caused by a fungus which seems to be identical with *Phytophthora cactorum* (Leb. et Cohn) Schroeter. Yet until the spring of 1922 this rot was apparently unknown to plant pathologists, a fact which may perhaps be taken as emphasizing the relatively slight amount of attention which has been given to diseases of this important fruit. Even to strawberry growers the disease was known merely as "water-soak," since they had observed that it was always most serious during wet weather. On the basis of facts to be presented later in the paper, it is proposed that this disease be known by the common name of "leather rot."

The writer first learned of the disease during the course of a trip through the strawberry producing sections of north-central Arkansas and western Tennessee in late April and early May, 1922. The rainfall during that time was unusually heavy and had been so for some time previous. The rot was found in every field inspected, the loss ranging from 10 per cent in some fields to 25 and even 50 per cent in others. Losses fully as heavy were noted the following year (1923) in the district around Beebe, Arkansas, and a few fields were seen in which for several days the loss amounted to three-fourths of the berries then ripening on the plants. The average loss for the whole season (1923) in this district was certainly not less than 20 per cent of the crop. In sections where it occurs, *Phytophthora* rot is one of the most serious diseases with which the strawberry grower has to contend and therefore deserves thorough investigation.

## EARLY FIELD OBSERVATIONS AND DESCRIPTION OF THE DISEASE

In all fields examined berries were found which were somewhat softened but tough and leathery and slightly browned—all of these being symptoms well known to the growers as typical of "water soak." After further investigation it seemed certain that they were caused by the attack of a parasite. Some fruits were seen which showed a white mold on the surface while still attached to the plant; others developed the mold after being held over night in a moist chamber. Mounts from the superficial growth showed almost always large numbers of conidia that were strongly suggestive of *Phytophthora* and cultures from affected fruits, whether moldy or not, yielded a white fungus; the fungus was later shown to be similar to and probably identical with *Phytophthora cactorum*, and its pathogenicity proved by inoculations.

In the field, *Phytophthora* rot (leather rot) affects strawberry fruits at all stages, from blossoming to full maturity. On all of them it is characterized by a definite though rather slight softening of affected tissues, by both external and internal discoloration and usually by a marked bitter taste; the tissues, however, become tough and leathery, so that the softening never even approximates the mushy, leaky condition produced by *Rhizopus* sp. nor the "soft rot" condition produced by *Pezizella lythri* (11).<sup>2</sup> The discoloration, particularly on the outside, varies so greatly that it must be described in detail.

<sup>1</sup> Received for publication Feb. 9, 1924.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 374-375.

In fruits that have barely begun to enlarge after petal fall, all parts, receptacle, stamens, calyx, and even the peduncle, become dark brown over affected areas; the brown, at the edges, shades off into the natural green of the unaffected portion. Fruits mature enough to have turned red show over affected areas the same yellow to light brown at the center of the spot but with a transition from this through darker brown to purple to the natural red of the berry; ripe, fully colored fruits show sometimes no color change at all except a slight darkening of the red over affected spots, or sometimes a faint tinge of the purple which forms the transition to red on less mature fruits.

On the market only the most advanced stages are seen. Here, however, the superficial growth of mold, rare in the field, is frequently seen and will serve as a diagnostic characteristic if taken in connection with the external color changes and the internal characteristics now to be described.

In cross and longi-section (Pl. 1) strawberries attacked by *Phytophthora* show a marked browning of the vascular system accompanied usually by a less intense browning of all the other tissues. In very early stages vascular browning is often the only visible symptom. So far as can be told by the eye or by touch, affected tissues are not disintegrated, though as mentioned earlier they are always softened and decidedly tough and leathery. At no time is there any clear line of demarcation between diseased and healthy flesh nor can a separation of the two be made by mechanical means; it is not possible to lift or scoop out the lesion as can so easily be done with the lesions of *Pezizella*.

### HISTORICAL REVIEW

This disease has been mentioned by the writer and his associates (13) in a preliminary report on field work. So far as can be learned that note and the present paper constitute the first record of *Phytophthora* as a parasite of strawberries in the United States. Osterwalder (6) found a *Phytophthora* on strawberries in Switzerland in 1912 which he identified as *P. omnivora*, apparently overlooking the fact that Schroeter (10 p. 235) in 1889 had adopted, instead of *omnivora*, the original specific name of *cactorum*, on the basis of the work of Lebert and Cohn (4) in 1870. The term *omnivora* had been used by de Bary in 1881 (see Wilson (16 p. 75) to include all members of the genus *Phytophthora* which were not referable to *infestans* and became in time a "waste basket" into which was thrown any unidentified *Phytophthora* (16). Wilson also makes the further statement in commenting on this record by Osterwalder and on other records by Marchal (5), Bubak (2) and Osterwalder (7) of the occurrence of *P. omnivora* on other hosts, that "The figures and descriptions indicate that more than one species of *Phytophthora* may be concerned and that in all probability none of these outbreaks were really due to the species which is credited with the damage." (See also Lafferty and Pethybridge (3), footnote, p. 35.)

### CAUSAL ORGANISM: APPEARANCE IN CULTURE

Pure cultures of the fungus were easily obtained from berries showing typical symptoms, by the following method: The berries were dipped twice in alcohol, the alcohol being burned off after each dipping; they were then cut across at the calyx end and the two halves were pulled apart with a flamed scalpel; portions of the infected "core" were then dug out from the torn surface with a freshly flamed scalpel and dropped into tubes of potato dextrose agar. More than 100 such plantings have been made, all but a very few of which gave the white, loosely matted growth characteristic of *Phytophthora* on this medium. The exceptions were either *Botrytis* or *Pezizella*. The fungus did not fill the tube though it occasionally grew up on the glass a centimeter or two above the surface of the agar slant. On oatmeal agar it usually made a scant, appressed growth, sometimes so scant that its presence could be told only by examination

with a hand lens. Old cultures sometimes showed a faint cream color but never turned brown and there was never any darkening of the medium. Cultures contaminated with bacteria were easily purified by inoculating them into apples and after six or eight days making a reisolation from the advancing edge of the lesion.

#### MICROSCOPICAL APPEARANCE

*Mycelium*.—The mycelium is coenocytic except when old. Hyphæ measure 4 to 7  $\mu$  in diameter, the average being about 6  $\mu$ . Young hyphæ are usually

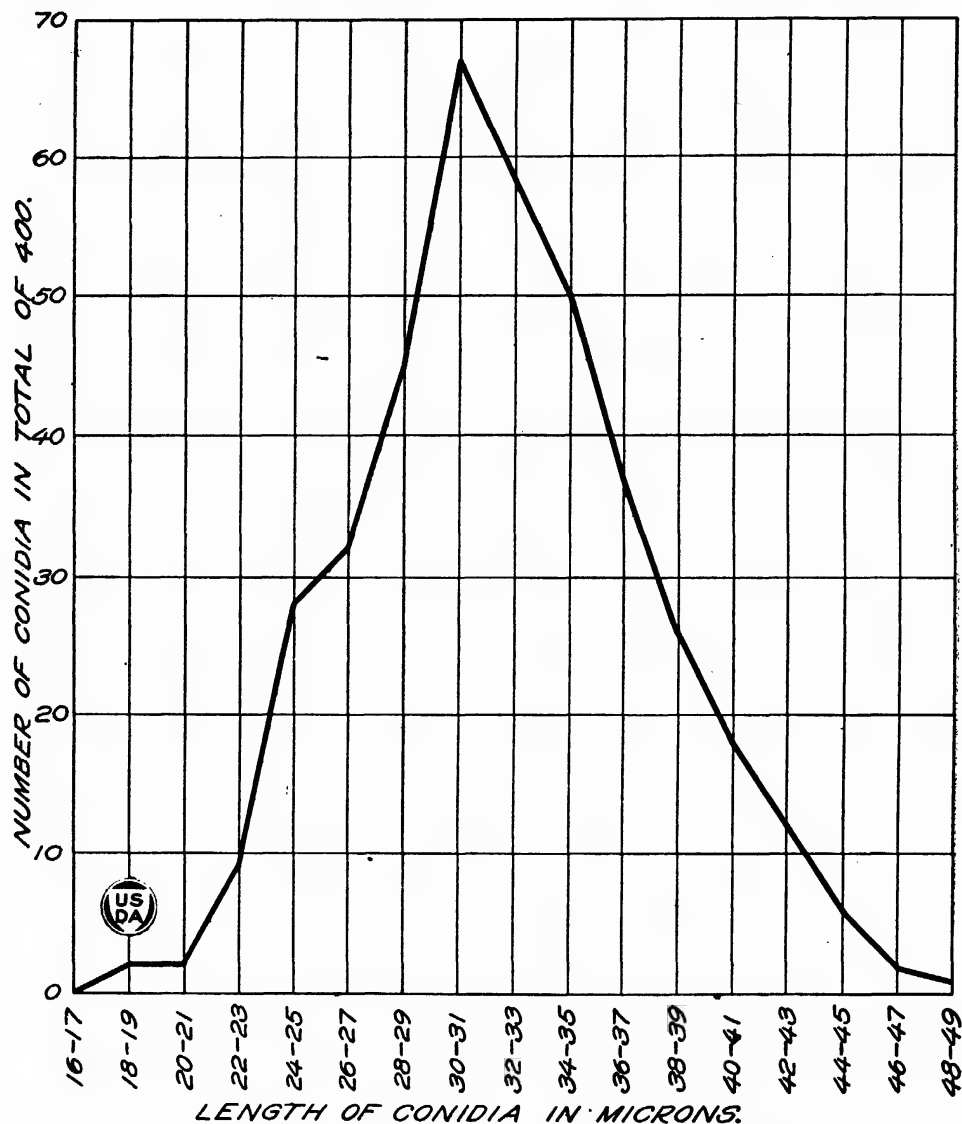


FIG. 1.—Graph showing the variation in length of conidia

full of protoplasm containing many oil drops, while old hyphæ are almost always practically empty.

*Asexual stage*.—In culture, conidia (Pl. 2, A, B) were produced freely at 10 to 20° C. but only in small numbers if at all, at higher temperatures. In the field they were found in abundance on fruits collected at Amite and Tickfaw, Louisiana, and at Beebe, Arkansas. Measurements of length and width of 400 conidia are given in Table I and are shown graphically in figures 1 and 2. The ratio of length to width of these conidia are given in Table II, and shown graphically in figure 3.

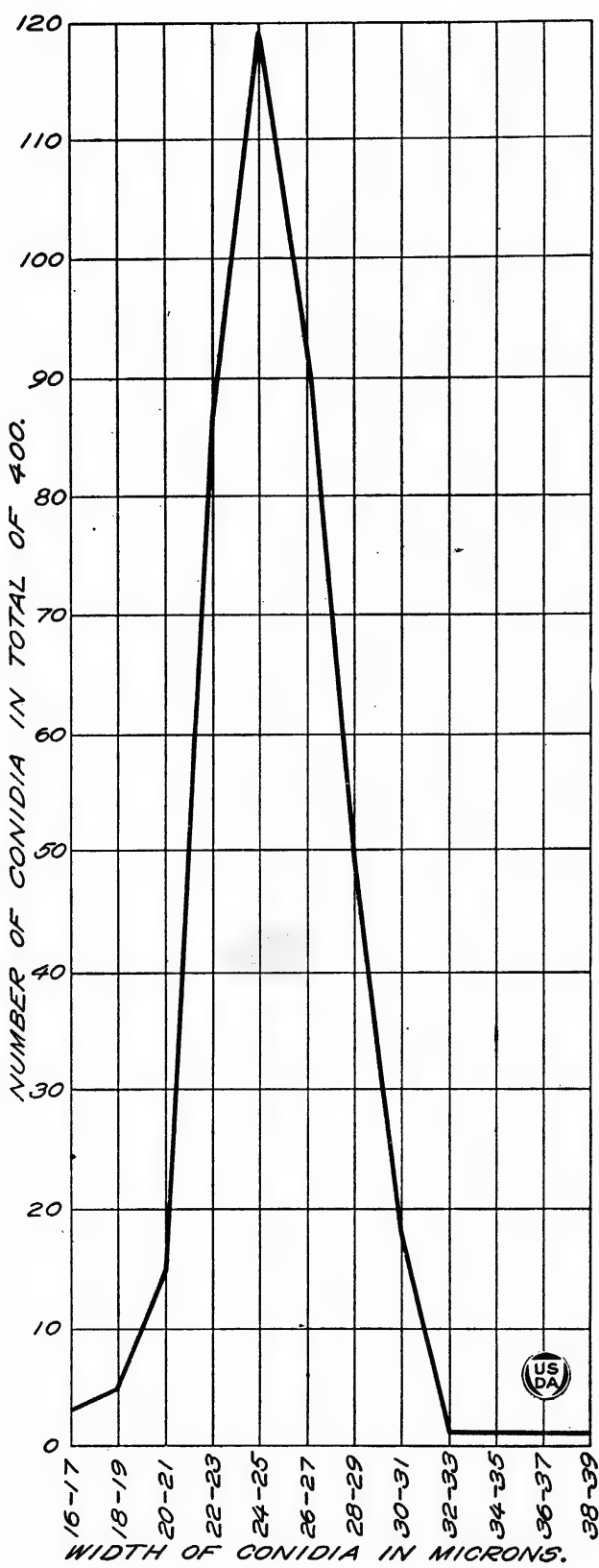


FIG. 2.—Graph showing the variation in width of conidia

TABLE I.—*Summary of measurements of conidia*

Class (in microns)	Number of conidia in each class ac- cording to—		Class (in microns)	Number of conidia in each class ac- cording to—	
	Length	Width		Length	Width
16-17.....		3	38-39.....	26	1
18-19.....	2	5	40-41.....	18	
20-21.....	2	23	42-43.....	1	
22-23.....	9	86	44-45.....	6	
24-25.....	28	119	46-47.....	2	
26-27.....	32	93	48-49.....	1	
28-29.....	45	49			
30-31.....	67	18	Total.....	400	400
32-33.....	64	1	Mean.....	32.26	25.09
34-35.....	50	1	Mode.....	30.31	24.25
36-37.....	37	1			

TABLE II.—*Arrangement in classes of the ratios of the length to the width of conidia, showing the limits of variation*

Class (in microns)	Number of conidia in each class	Class (in microns)	Number of conidia in each class
1.00-1.04.....	12	1.50-1.54.....	16
1.05-1.09.....	22	1.55-1.59.....	6
1.10-1.14.....	34	1.60-1.64.....	5
1.15-1.19.....	56	1.65-1.69.....	4
1.20-1.24.....	63	1.70-1.74.....	2
1.25-1.29.....	52		
1.30-1.34.....	44	Total.....	400
1.35-1.39.....	25	Mean.....	1.24
1.40-1.44.....	36	Mode.....	1.20-1.24
1.45-1.49.....	23		

Conidia from the Louisiana material discharged zoospores within five minutes after mounting in water on a slide, while those from Arkansas material failed to produce zoospores at any time even after repeated trials. Material from both sources were examined within an hour after collection.

Both in culture and on berries collected in the field the conidia were found germinating in the direct manner, either by a simple germ tube or by the formation of secondary and even tertiary conidia (Pl. 2, C to G). In mounts from a few cultures these secondary and tertiary conidia were found discharging zoospores.

The manner of zoospore discharge, for all classes of conidia, is the same as that already described (8, 9) for *P. cactorum* and other *Phytophthora* species; that is, there is first a bulging of the papilla and a movement outward of the already differentiated zoospores. The first three or four move out so quickly that they seem almost to burst out, though they go singly through the narrow passage or neck at the tip of the conidium. All of them assume an hour-glass shape as they pass through the neck. The first few that come out are held together for a few seconds by the vesicle formed by the bulging out of the papilla but finally separate—presumably when the vesicle breaks, though the actual breaking was not seen—remain quiescent for a few seconds more and then swim rapidly away. The zoospores that follow the first lot come out more slowly, although the total time for what might be called normal discharge is about thirty seconds. In fact, it is almost certain that if the zoospores do not all come out within that time some of them will not come out at all. Those that remain within the conidium frequently germinate there, the germ tubes growing out through the conidium wall.



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22-23.....	9	86	44-45.....	6	-----
24-25.....	28	119	46-47.....	2	-----
26-27.....	32	93	48-49.....	1	-----
28-29.....	45	49			
30-31.....	67	18	Total.....	400	400
32-33.....	64	1	Mean.....	32.26	25.09
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1.15-1.19.....	56	1.65-1.69.....	4
1.20-1.24.....	63	1.70-1.74.....	2
1.25-1.29.....	52		
1.30-1.34.....	44	Total.....	400
1.35-1.39.....	25	Mean.....	1.24
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1.45-1.49.....	23		

Conidia from the Louisiana material discharged zoospores within five minutes after mounting in water on a slide, while those from Arkansas material failed to produce zoospores at any time even after repeated trials. Material from both sources were examined within an hour after collection.

Both in culture and on berries collected in the field the conidia were found germinating in the direct manner, either by a simple germ tube or by the formation of secondary and even tertiary conidia (Pl. 2, C to G). In mounts from a few cultures these secondary and tertiary conidia were found discharging zoospores.

The manner of zoospore discharge, for all classes of conidia, is the same as that already described (8, 9) for *P. cactorum* and other *Phytophthora* species; that is, there is first a bulging of the papilla and a movement outward of the already differentiated zoospores. The first three or four move out so quickly that they seem almost to burst out, though they go singly through the narrow passage or neck at the tip of the conidium. All of them assume an hour-glass shape as they pass through the neck. The first few that come out are held together for a few seconds by the vesicle formed by the bulging out of the papilla but finally separate—presumably when the vesicle breaks, though the actual breaking was not seen—remain quiescent for a few seconds more and then swim rapidly away. The zoospores that follow the first lot come out more slowly, although the total time for what might be called normal discharge is about thirty seconds. In fact, it is almost certain that if the zoospores do not all come out within that time some of them will not come out at all. Those that remain within the conidium frequently germinate there, the germ tubes growing out through the conidium wall.

From a study of numerous mounts of conidia ripe for germination it was found that the average time for discharge to begin is about five minutes after the mount is made. After discharge the zoospores are usually active for about half an hour but finally come to rest and within 15 to 30 minutes later have germinated, the total time from discharge to germination being roughly one hour. In mounts made with a cover glass it was noted that the zoospores, whether germinating or still active, were most numerous around the edges of the cover glass, presumably

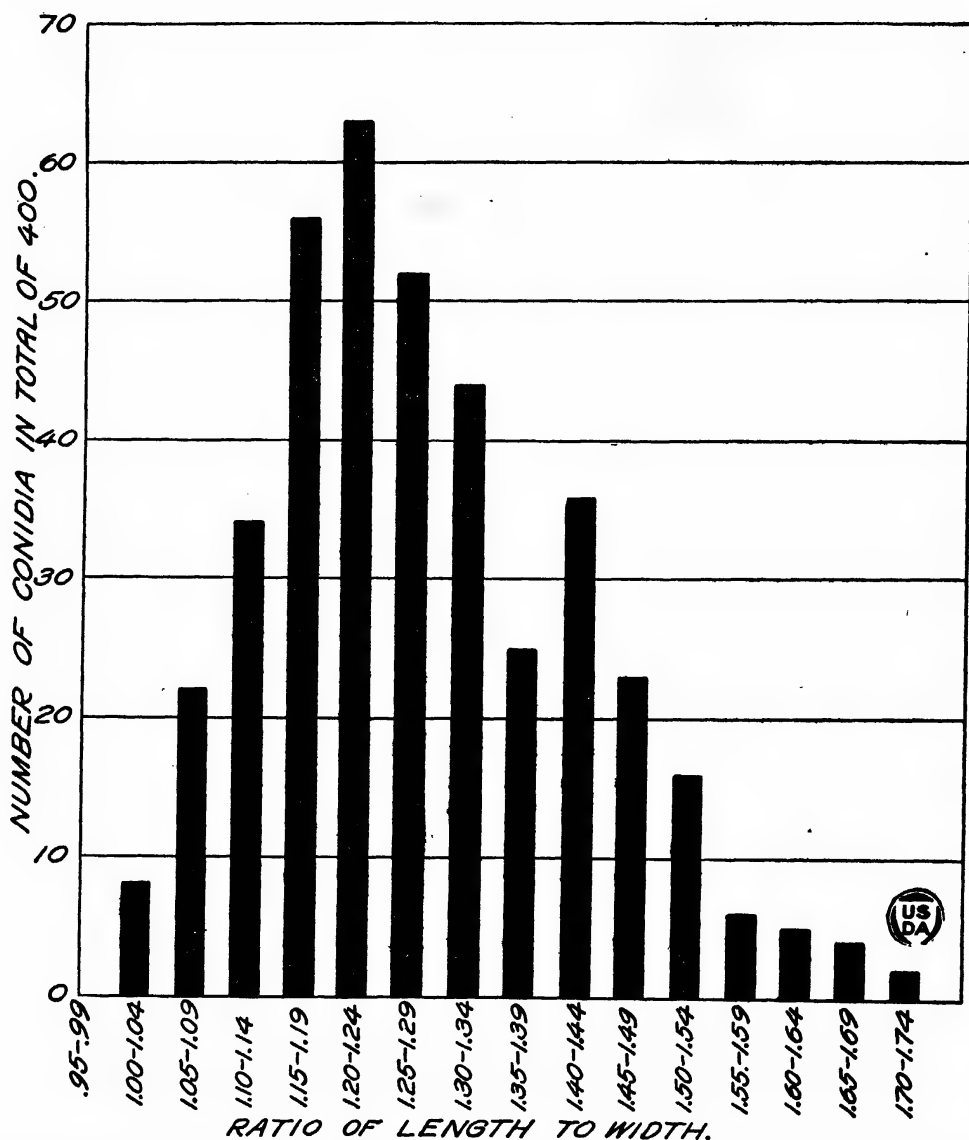


FIG. 3.—Arrangement in classes, of the ratios of the length to the width of conidia, showing the limits of variation

because of the greater supply of oxygen there. It was noted also that zoospores from conidia produced on berries in the field were much more active than those from conidia produced in cultures. The number of zoospores produced by a conidium varies from 4 to 30, the average being 15 to 18. While still active the zoospores are kidney-shaped and have two cilia; soon after they come to rest they become circular in outline and the cilia can no longer be detected. For figures illustrating the zoospores, their discharge and germination, the reader is referred to Beach (1, fig. 25) and Rosenbaum (8, fig. 18).

**SEXUAL STAGE.**—The sexual stage was produced in abundance on potato dextrose agar, oatmeal agar, and strawberry extract agar<sup>3</sup> at all temperatures from 8 to 25° C. and at the higher temperatures frequently within five days after the culture was made. It was also found on naturally infested fruits collected in the field.

Observations to date indicate that the antheridia are practically always paragynous. (Pl. 2, H to M). In old cultures on oatmeal paste a few were seen which were plainly amphigynous. The sexual organs frequently arise from the same hypha (Pl. 2, H, I, K), though they sometimes seem to arise from different hyphae (Pl. 2, J, L, M). It is possible, however, that such hyphae are merely branches having a common origin, which can not be found because the connection was broken or the hypae became badly entangled when the mount was made. The question is one which deserves further investigation.

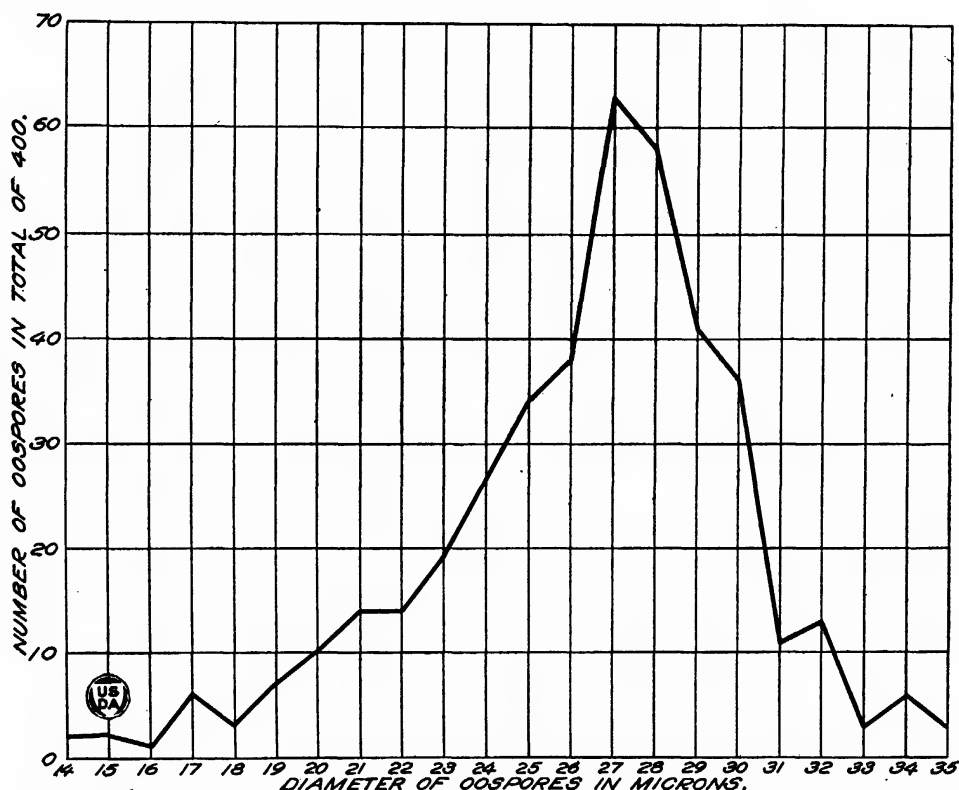


FIG. 4.—Graph showing the variation in diameter of oospores

Oogonia are hyaline, mature oospores faint yellow to sometimes a delicate orange. Germinating oospores, one of which is shown in Plate 2, N, have been observed a few times in old cultures on oatmeal agar.

Measurements of 400 oospores made on material from oatmeal agar cultures held at room temperatures for 20 days, are given in Table III, and shown graphically in figure 4.

**SPHAERO-CONIDIA.**—In cultures 4 to 6 months old on potato dextrose agar a few bodies have been seen which resemble the sphaero-conidia described and figured by Lafferty and Pethybridge (3, p. 37, and Pl. 2, fig. 13) for *P. cactorum* and *P. fagi*. These bodies vary in diameter from 26 to 44  $\mu$  and average about 33  $\mu$ . They have not been seen on diseased berries.

<sup>3</sup> The strawberry extract agar consisted of 1,000 cc., of water, 15 gm. of agar and the expressed juice from 2 quarts of ripe strawberries. When titrated it was found to have a reaction of +31, Fuller's scale.

TABLE III.—Summary of measurements of oospores

Class (in microns)	Number of spores in each class	Class (in microns)	Number of spores in each class
14.....	2	27.....	63
15.....	2	28.....	58
16.....	1	29.....	41
17.....	6	30.....	36
18.....	3	31.....	11
19.....	7	32.....	13
20.....	10	33.....	3
21.....	14	34.....	6
22.....	14	35.....	3
23.....	19		
24.....	16	Total .....	400
25.....	34	Mean .....	26.42
26.....	38	Mode.....	27.0

The above description and measurements are believed to establish it as a fact that the organism dealt with in this paper is *Phytophthora cactorum*, since they agree so closely with those already published for that fungus by other workers (1, 8, 10). The finding of both paragynous and amphigynous antheridia in cultures of the strawberry *Phytophthora* is still further evidence that the organism is *P. cactorum* since Lafferty and Pethybridge (3) have reported the discovery of both kinds of antheridia in cultures of *P. cactorum* obtained by them from various sources.

INOCULATION EXPERIMENTS

Two methods of inoculation were used, one of them in the laboratory at the University of Chicago, the other in the field laboratory at Beebe, Ark. Procedure in the former was as follows:

Berries apparently free from disease were selected either from quart boxes purchased on the market (out of crates stamped "Klondike," "Haverland" or "Dunlap") or were picked from a small patch of Progressive, an everbearing variety, to which the writer had access. These berries were washed three or four times with sterile water, placed in sterile glass moist chambers in lots of 20 to 25 and inoculated by forcing a small fragment of mycelial growth from a pure culture down into the flesh. They were then covered up, but in such a way as to allow some ventilation, since it was found that too close covering kept the berries so moist that *Botrytis* on northern-grown berries and *Rhizopus* on berries from all sections frequently caused excessive decay before *Phytophthora* got well started.

Within three days, at 20 to 25° C., berries inoculated by this method, if they had escaped *Rhizopus* (or *Botrytis*), showed vascular browning and slight softening; within four days they showed a marked browning of the surface and had reached the tough leathery condition described earlier in this paper. For some unknown reason they did not have quite the appearance of berries naturally infected in the field, though they always gave *Phytophthora* when plantings were made from them, under aseptic conditions, into agar tubes. They were atypical externally, in that they did not show the color pattern or color transition, from pale yellow to brown to purple to red.

The same is true of berries inoculated by the second method, which, nevertheless, gave practically 100 per cent infection by *Phytophthora*. These berries (varieties Klondike and Aroma) selected as before as free or apparently free of disease, were washed with sterile water as soon as they were brought in from the field; 20 to 25 of them were then placed on clean, but not sterilized, newspaper, in about a quarter of an inch of water in pans, with five or six moldy "water-soaked" (leather rot) berries scattered among them. The experiment was al-

lowed to stand for an hour, with the berries exposed to the light; that is, for the length of time, and under the conditions, which make possible the discharge and germination of zoospores. The healthy berries were then removed, placed on clean moist newspaper in a sterilized pan and covered with another pan. Within three days the few that were taken out and cut showed softening and vascular browning; after one or two days more the remaining berries were covered with a scant growth of white mold and plantings from them yielded only *Phytophthora*.

In other words, the berries became infected under conditions in which infection by zoospores was not only possible but actually much more probable than infection, for example, by floating bits of mycelium. It is true, as noted earlier, that no zoospores were seen in any of the mounts made from Arkansas material. Yet, in the absence of proof to the contrary, this failure to find zoospores must be ascribed to a merely accidental failure to find conidia that were ripe for discharge.

From these experiments (second method) and from control experiments conducted in the same way, except for the diseased berries, it was necessary at the end of 24 hours to remove a few fruits that showed signs of leather rot, the result of having to use berries from a section of the country in which the rot was so common that it was likely to occur in incipient stages even in fruit selected with the utmost care. The remaining control fruits and controls wounded but not inoculated with mycelium (Chicago experiments) failed to give *Phytophthora* at any time, though by the end of five or six days 75 to 90 per cent of them were plainly diseased. Plantings from these diseased control fruits gave *Pezizella*, *Sphaeronemella*, an unidentified *Rhizoctonia*, and *Botrytis*. In experiments conducted in the field laboratory, *Rhizopus* gave very little trouble, probably because the berries came direct from the field and had been bruised only slightly or not at all.

Attempts to inoculate strawberries with *Phytophthora* by laying bits of an agar culture of the fungus on the surface of uninjured fruits were always unsuccessful.

### INFECTION IN THE FIELD

Nothing definite is known about how infection takes place in the field. It may be that under field conditions the mycelium of *Phytophthora* is better able to enter uninjured fruits than it has appeared to be in the laboratory. It is certain that except after severe storms or general insect attack very few berries in the field have skin breaks of any sort, not to mention such wounds as were made in berries used for inoculation experiments. But conditions in the field which favor leather rot are precisely those which in the laboratory favor the discharge and germination of zoospores. It seems likely therefore that most of the field infection is caused by zoospores; whether all of it is so caused is matter for further investigation. Berries which touch the ground are always first affected, or show the disease first, on the lower side; it is not known, however, whether they become infected by zoospores or by direct growth of mycelium from the soil. This statement, in turn, raises the question whether the *Phytophthora* dealt with in this paper is a true soil fungus, in strawberry fields, or whether it lives on debris on the soil surface. Berries which hang free in the air and which may show the disease either at the tip or anywhere on the sides, could have become infected by zoospores or mycelium spattered onto them during rains; they might equally as well have become infected by means of zoospores which moved from the soil along the fruit stem to the fruit through the surface film of water which, during wet weather, covers all parts of the plant for hours at a time. More investigation is needed on all of these points.

Investigation is also needed on the relation of the calyx to rot of the fruit. Even under conditions of moderate rainfall and temperature there are always

to be found in the strawberry districts of the South, a few berries on which the calyces are discolored (brown or gray) and plainly diseased. In warm, wet weather the condition may become quite general. All varieties may show it but Klondike usually suffers most severely. Observations, both in the field and on the market, during the seasons of 1922 and 1923, have shown that diseased calyces are often associated with disease of the berry itself. Many berries have been found which showed signs of *Phytophthora* infection at the place where an apparently diseased calyx lobe lay in contact with the fruit surface. In such specimens a cross or longitudinal section always disclosed the typical vascular browning; plantings from affected areas always developed *Phytophthora*.

Many other fruits, on which the whole calyx was diseased, showed infection at the top in a ring or zone surrounding the base of the calyx. Plantings from this infected zone, in different berries, gave *Phytophthora*, *Rhizoctonia* sp. or *Pezizella*, or sometimes only bacteria. No cultures have been made from diseased calyces nor is there any proof that infection actually spreads from the calyx down into the fruit. The presumption is strong, however, that it very often does, or in other words, that the calyx when diseased is a source of danger to the fruit. One is reminded in this connection of the much more striking case of stem-end rot of citrus fruit, which, according to Winston, Fulton, and Bowman, (17) is probably due almost entirely to growth of either *Diplodia* or *Phomopsis* from the diseased calyx into underlying tissues of the fruit.

### SUSCEPTIBLE VARIETIES

Under field conditions leather rot has been seen mainly on the varieties Klondike and Aroma, and always is much more serious on the former than on the latter. Gandy, Missionary, and Lady Thompson appear to be quite resistant if not immune. Klondike is the sole commercial variety in Louisiana and one of two, Aroma being the other, in Arkansas, Kentucky, and Tennessee; Aroma is the main variety in southwest Missouri (Monett-Neosho district) and is of considerable importance in southern Illinois. Gandy is grown commercially in southern Illinois, and Lady Thompson to a small extent in Arkansas and Tennessee. In the States discussed in this paper, the variety Missionary is grown very little and was actually seen in only one field in White County, Ark.

### RANGE OF THE DISEASE

Leather rot was first observed by the writer on Klondike strawberries at Judsonia, Ark., during the first week of May, 1922, and again a few days afterward at Humboldt, Tenn. During the last week of May and the first two weeks of June it was found on the market in Klondike and Aroma strawberries from Bald Knob, Ark., from Monett, Mo., and from a small producing section near St. Louis, Mo. During the strawberry season of 1923 it was seen in the field at Gulfport, Miss., Amite and Tickfaw, La. (in the Hammond district), and at Beebe and McRae, Ark.; on the market (after June 1) it was seen in berries from southern Illinois and the Bowling Green section of Kentucky. Judsonia, Bald Knob, Beebe, and McRae, Ark., are all in White County and there is every reason to believe that the disease could have been found in practically all Klondike and Aroma strawberry fields in the county if there had been time to make the survey. It probably occurs also throughout the strawberry districts of west Tennessee, and not merely in the district around Humboldt. The writer has neither visited producing sections of east Tennessee nor seen strawberries from there but on the basis of information furnished by E. E. Conklin, supervising inspector for the United States Department of Agriculture at Cleveland, Tenn., during the strawberry shipping season of 1923, he is inclined to believe that the disease occurs in east Tennessee also. It is possible that the "lilac soft rot" reported by

Sherbakoff (12) from Tennessee is identical with leather rot, although Sherbakoff states that isolations from this "soft rot" gave a Pythium. The records for Monett, Mo., and for southern Illinois are based on one carload from each State, the only carloads from those States in which the rot was seen either in 1922 or 1923. This of course does not indicate heavy loss. There is other evidence also that in those States the disease is much less serious than in States farther south. But even if only the authentic records of occurrence are considered it is apparent that leather rot is rather widely distributed through the South in States which in 1922 (see fig. 5 and Table IV) were the leaders in strawberry production in the United States. Its relation to production and marketing of the crop will be brought out in the subsequent discussion.

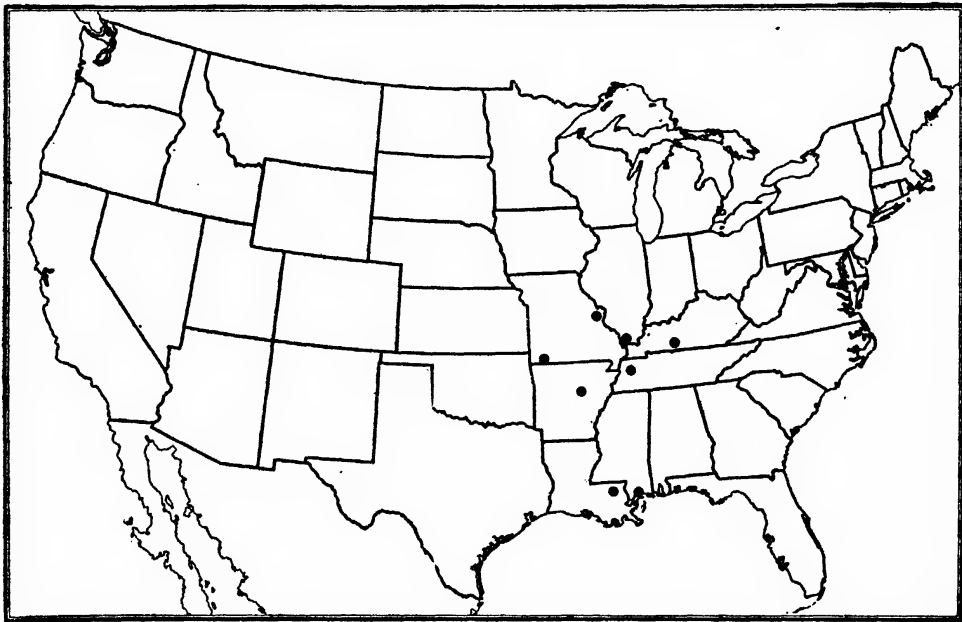


FIG. 5.—Distribution of leather rot of strawberries in the United States as determined during the picking seasons of 1922 and 1923 by inspection of fruit in the field and of shipped fruit on the market. The States and counties represented are as follows: Louisiana, Tangipahoa County; Mississippi, Harrison County; Arkansas, White County; Tennessee, Gibson, Bells, and Crockett Counties; Missouri, Newton, Barry, and Jefferson Counties; Kentucky, Warren County; Illinois, Union and Pulaski Counties

TABLE IV.—Carlot shipments of strawberries by States of origin, 1922 <sup>1</sup>

Tennessee .....	3, 592	Kentucky .....	756
Arkansas .....	2, 190	Michigan .....	650
Missouri .....	2, 043	Alabama .....	459
Virginia .....	1, 670	New York .....	330
Maryland .....	1, 629	Florida .....	325
Louisiana .....	1, 540	New Jersey .....	274
North Carolina .....	1, 101	Illinois .....	260
Delaware .....	940	All other States .....	789

RELATION OF LEATHER ROT TO RAINFALL

Strawberry growers in the region dealt with in this paper have observed that "water soak" (leather rot) is worse during wet weather than during dry weather. Their use of the term implies a belief, often explicitly stated, that the berries

<sup>1</sup> Figures taken from the Yearbook of the United States Department of Agriculture for 1922 (14, p. 772)

simply take up too much water and become worthless. As a matter of fact this probably does happen with some of the fruit, during wet weather. It is well known that strawberries produced during such weather are likely to be soft, succulent, and easily damaged even by the most careful handling. It is entirely possible that the local name of "water soak" is sometimes applied to berries that are not infected by any parasite but have merely been rendered soft and mushy by too much rain. It has been shown, however, that much of the condition known to the growers as "water soak" is actually the disease caused by *Phytophthora*. On the other hand, and in full agreement with observations by the growers, it has been found that there is a close relation between leather rot and rainfall. The fact that this relation exists was established in a general way by the writer, in 1922, and more definitely, though with qualifications, during the season of 1923 in connection with the holding tests and other work on strawberry diseases carried on at Beebe, Ark.

It is worth noting here that in the district around Hammond, La., where rainfall during the picking season is at least as heavy as in important strawberry districts farther north, leather rot occurs only rarely. It has been observed moreover by growers in the more northern districts that the condition often prevails when rainfall has not been excessive but merely more or less continuous for several days.

In the holding tests, made at intervals during the period from May 5 to 31, 2 to 8 quart lots of berries from 30 experimental plots and from various other fields in the district, were held for four days in the local cold-storage plant at a temperature of 40 to 45° F. The filled quarts were used just as they came from the pickers, or from crates ready for shipment at the loading platform. The diseased or rotten berries that would have been removed by resorting certainly amounted at all times to less than 1 per cent.

TABLE V.—Percentages of (1) leather rot, (2) other rots, (3) all rots, in strawberries used in holding tests, on first day after removal from storage, Beebe, Ark., 1923

Kind of rot	Dates in May when picked—													
	5	7	8	9	11	14	15	16	17	18	19	21	23	26
Leather rot---	1.3	0.0	3.1	3.0	41.5	4.0	5.1	29.2	45.4	68.5	10.9	6.2	16.1	25.1
Other rots----	5.0	7.2	2.2	6.5	11.7	.2	2.5	.9	3.3	.0	.4	2.9	31.7	27.5
All rots--	6.3	7.2	5.3	9.5	53.2	4.2	7.6	30.1	48.7	68.5	11.3	9.1	47.8	52.6

At the end of the four days the various lots were removed and the berries were carefully hand sorted and counted to determine the amount and kind of rot present. Except where noted, the count was made immediately after the fruit was removed from cold storage. A summary of results obtained in these tests is given in Table V. The various rots were determined by touch and sight, these being checked occasionally by cutting diseased fruits or making cultures from them. Figure 6 is based on these counts and shows graphically the relation of rot to weather conditions, particularly rainfall. The rainfall record was obtained by means of a gauge loaned by the United States Weather Bureau at Little Rock. Since there was no accurate local record, the temperature data used are those recorded by the Weather Bureau (15) for Little Rock, Ark., which is about 35 miles from Beebe and considerably nearer to it than are any of the other weather stations in that part of the State.



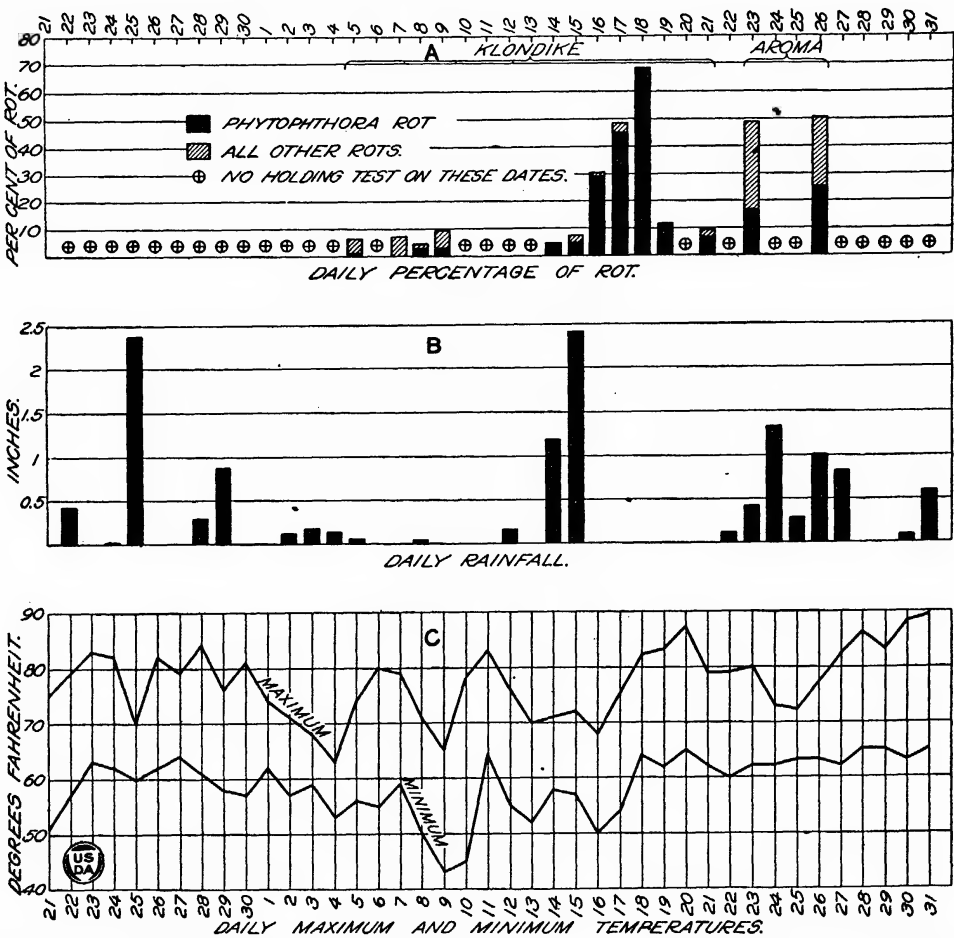


FIG. 6.—A. Percentage of rot found immediately after removal from storage, in various lots of strawberries held for four days at a temperature of 40 to 45° F. Percentages are entered for the day on which the berries were picked. B. Daily rainfall in inches. Record of rainfall was kept at Beebe, Ark., by means of a standard rain gauge loaned by the United States Weather Bureau. C Daily maximum and minimum temperatures, as recorded by the United States Weather Bureau at Little Rock, Ark., about 35 miles from Beebe.

TABLE VI.—Daily temperatures, April 21 to May 31, Little Rock, Ark., and rainfall for the same period, at Beebe, Ark.

Date	Temperature (degrees F.)		Rainfall	Date	Temperature (degrees F.)		Rainfall
	Maxi- mum	Mini- mum			Maxi- mum	Mini- mum	
			Inches				Inches
Apr. 21	75	51	0.41	May 12	76	55	0.15
22	79	57	.00	13	70	52	.01
23	83	63	.01	14	71	58	1.20
24	82	62	1.15	15	72	57	2.40
25	70	60	.00	16	68	50	.00
26	82	57	.00	17	75	54	.00
27	79	64	.30	18	82	64	.00
28	84	61	2.38	19	83	62	.00
29	76	58	.86	20	87	65	.00
30	81	57	.00	21	79	62	.00
May 1	74	62	.00	22	78	60	.11
2	64	57	.13	23	79	62	.47
3	68	59	.18	24	73	62	1.33
4	63	53	.15	25	72	63	.27
5	74	56	.06	26	77	63	1.00
6	80	55	.00	27	82	62	.80
7	79	59	.00	28	86	65	.00
8	71	50	.06	29	83	65	.00
9	65	43	.00	30	88	63	.08
10	78	45	.00	31	89	65	.58
11	83	64	.00				

The rot percentages are entered, on figure 6, not for the day on which the first counts were made but for the day on which the fruit was picked; they actually represent the rot which had developed four days after picking. The most striking portion of the chart is that which covers holding tests with Klondike strawberries for the period from May 14 to May 21, inclusive; during the first two days of that period there was heavy rainfall—on the 14th, 1.20 inches; on the 15th, 2.40—and on the remaining four days no rain at all. The temperature for the six days, as recorded at Little Rock, ranged from 68 to 83° F. for the maximum and from 50 to 65° F. for the minimum, with the higher readings coming in the latter half of the period. General notes on weather show that four of the days were cloudy, two clear.

*Weather notes, Beebe, Ark., from May 5 to May 31, inclusive*

May 5—Cloudy.	May 19—Clear.
May 6—Clear.	May 20—Clear.
May 7—Rain forenoon, partly clear afternoon.	May 21—Partly cloudy; rain at night.
May 8—Partly cloudy.	May 22—Partly cloudy; rain at night.
May 9—Clear.	May 23—Partly cloudy; warm in after- noon.
May 10—Clear, south wind.	May 24—Cloudy, showers.
May 11—Cloudy.	May 25—Cloudy, showers.
May 12—Cloudy, rain afternoon.	May 26—Cloudy, rain afternoon.
May 13—Clear; north wind; rain at night.	May 27—Clear.
May 14—Cloudy.	May 28—Clear.
May 15—Cloudy.	May 29—Mostly cloudy.
May 16—Cloudy.	May 30—Clear, rained late afternoon; picking practically stopped.
May 17—Partly cloudy.	May 31—Clear.
May 18—Clear.	

It is apparent that during these six days there was excellent opportunity for determining what correlation, if any, exists between rainfall and leather rot of strawberries. Reference to the chart shows that in this instance positive correlation does exist. For fruit picked on the 14th and 15th, the days when the rain occurred, the percentage of rot was about what it had been during the previous 10 days. (This statement is based partly on what shows on the chart and partly on field observations and counts.) But for the fruit picked on the 16th and counted four days later the percentage of rot rose to 29.2 per cent, on the 17th to 45.4 per cent, and on the 18th, four days after the first rain, to a maximum of 68.5 per cent; on the 19th it dropped back to 10.9 per cent and on the 21st to 6.2 per cent. (Compare 3 per cent on the 9th and 4 per cent on the 15th.) This course of events is practically that observed in inoculation experiments, where the first visible symptoms of leather rot appeared on the third day after inoculation and fully developed rot on the fourth. It also helps to explain reports by representatives of the Federal Market News Service, by receivers on the markets, and by the sales manager of the local association at Beebe that Klondike berries picked and shipped on the 18th showed more loss on arrival at market than did those shipped on any other day from the 14th to the 21st.

The marked drop in rot from 68.5 per cent on the 18th to 10.9 on the 19th and 6.2 on the 21st is best explained by assuming that very little infection occurred after the 15th, the last day of rainy weather. It is exactly what should be expected if *Phytophthora* infection occurs only in wet weather and takes three to four days to become visible rot. If, however, there had been rains for several days or only two or three days apart, the attacks would have so overlapped as

to obscure the rise and fall in amount of rot due to any one of them. Under such conditions it might easily have seemed, as it actually has to growers many times, that rot on any given day had resulted from the weather of that or the preceding day. When the course of one attack, as in the instance under discussion, can be followed from beginning to end, the lag in occurrence of the maximum as well as the duration of the attack both become very apparent.

Under this topic one more point remains to be covered. As mentioned earlier, growers have observed that leather rot often occurs when rainfall is not heavy but merely more or less continuous for several days. It is therefore worth noting that the rot did occur in just such weather during the course of the work here described. The four days from May 2 to May 5 were rainy, but the heaviest rainfall on any one day was less than 0.2 inch. There was also a rain of 0.06 inch on the 8th. Yet during the five days from May 5 to 9, the first counts being made on the 5th, leather rot was found as follows:

	In the field	In holding tests
May 5	No counts.....	1.3.
6	do.....	No counts.
7	do.....	0.0.
8	8.0.....	3.1.
9	5.0.....	3.0.

RELATION OF LEATHER ROT TO FIELD TEMPERATURES AND DEGREE OF CLOUDINESS

The relation of leather rot to other elements of the weather besides rainfall can not be so easily determined. Laboratory findings and field observations justify certain assumptions but are not yet complete or extensive enough to be used as a basis for conclusions. For the period from May 14 to 21 general cloudiness and fairly low temperatures during the first part of the period prolonged the time during which both soil and plants were covered with a film of moisture. This, in turn, by favoring the release and distribution of zoospores, would presumably increase the amount of rot and lengthen the time during which the rot would be heavy. But on the other hand, higher temperatures during the latter part of the period would hasten development of the rot in infected berries and so bring the attack to an end sooner, provided there was no further rainfall. They might thus account, in part at least, for the sharp drop from 68.5 per cent of rot on the 18th to 10.9 per cent on the 19th. It is a fact, noted both by the writer and by many growers, that during hot weather three or four days after a rain leather rot develops and becomes visible very rapidly. On the 18th and again on the 26th, 28th, and 29th, all of which had maximum temperatures of 77 to 85° F., numerous packed crates were seen in which the berries picked in the morning as being apparently sound had by afternoon developed so much visible *Phytophthora* rot that they had to be thrown away.

PROPORTION OF LEATHER ROT TO OTHER ROTS

IN HOLDING TESTS.—During the six-day period mentioned above, the percentage of rots other than leather rot (mostly *Pezizella* and *Rhizoctonia* sp.) in cold storage lots of Klondike berries, showed no progressive increase from day to day. In fact, on the 18th when leather rot was the highest, no other rots were found. During the period from May 5 to 14 there was no progressive increase in any of the rots, probably because rainfall was light and percentage of all rots low. On the other hand, there is evidence, furnished, it is true, by only two holding tests

(fig. 6), that on Aroma berries during the later period of rainy weather, May 22 to 27, leather rot increased after the rain and that as it increased other rots decreased. Owing to press of other work, the first count on the two tests had to be postponed until about 30 hours after removal of the fruit from storage. Percentage of all rots is therefore excessively high, and not strictly comparable with the percentage found when counts were made immediately after removal from storage. Reference to figure 6 and Table VIII, May 23 and 26, shows nevertheless that on both of these dates, rots other than that caused by *Phytophthora* constituted a much larger proportion of the total rot, on the variety Aroma, than they did during the preceding week on the variety Klondike. But according to evidence already adduced, the rainfall from May 22 to 27 was very favorable for the development of leather rot. The obvious explanation, and one which is supported by observations and counts made in the field, is that the variety Aroma is less susceptible than the variety Klondike to leather rot.

IN THE FIELD.—Incomplete data on occurrence of the various rots in the field are given in Table VIII. These figures indicate that under field conditions there is for both Aroma and Klondike, a progressive increase in *Phytophthora* rot as the season advances and a corresponding decrease in other rots; for Aroma, a preponderance of "other rots" over leather rot about as great as that noted in the holding tests.

TABLE VIII.—Percentage of (1) leather rot, (2) other rots, occurring in strawberry fruits in fields, near Beebe, Ark., May, 1923.<sup>a</sup>

Date	Leather rot	Other rots	Variety
May 8.....	19.1	80.9	Klondike.
May 9.....	26.6	73.4	Do.
May 15.....	88.4	11.6	Do.
May 16.....	91.2	8.8	Do.
May 25.....	14.8	85.2	Aroma.
May 26.....	35.0	65.0	Do.
May 28.....	55.0	45.0	Do.

<sup>a</sup> Percentages calculated on the basis of total rot as 100 per cent.

TABLE IX.—Percentage of (1) sound, and (2) rotten strawberries in fields near Beebe, Ark., May, 1922

Date	Sound	Rotten	Variety
	<i>Per cent</i>	<i>Per cent</i>	
May 8.....	62	38	Klondike.
May 9.....	82	18	Do.
May 16.....	90	10	Do.
May 18.....	76	24	Do.
May 25.....	60	40	Aroma.
May 26.....	25	75	Do.
May 29 <sup>a</sup> .....	33	67	Do.

<sup>a</sup> The figures for May 29 are the average of an estimate, by three competent observers, of the loss from rot on May 28 and 29 in the district immediately surrounding Beebe; all other figures in the table are based on field counts made on the date opposite which they are entered.

RELATION OF STRAWBERRY PHYTOPHTHORA TO TEMPERATURE  
IN LABORATORY EXPERIMENTS AND IN HOLDING TESTS

IN LABORATORY EXPERIMENTS.—The effect of various temperatures on the growth of the fungus (in culture) was tested by holding sets of three plates (of potato dextrose agar) inoculated with the organism, in each of nine compartments of an Altmann constant temperature apparatus. At the end of 10 days the plates were removed and the diameter of the growth was measured. Results of one such experiment gave the following results:

Average temperature °C.....	1.0	3.6	8.2	11.8	12.6	14.7	15.4	17.6	19.1
Diameter of growth in mm.....	0.0	0.0	3.5	4.4	5.6	6.2	6.9	7.5	8.0

The growth at room temperature, 20 to 25°C. averaged 8.6 for the 10 days. At 36°C. there was no growth at all. Evidently, therefore, the optimum for growth lies somewhere between 25 and 36°C. though no tests were run at these intermediate temperature, since they were not obtainable at the time the above test was made. The growth at 8.2°C. was a flat, dense surface mat; at higher temperatures the growth was more aerial and fluffy, both of these characters becoming more pronounced with each successive rise in temperature. At all temperatures where growth occurred the fungus grew both on the surface of and down into the agar.

IN HOLDING TESTS.—As already noted, the fruit used for holding tests was apparently healthy when stored. Yet after four days' storage at 40 to 45°F. all 14 lots of this fruit showed 4 per cent or more of rot (Table VIII) and six of them 25 to nearly 70 per cent. The rots found on the first day after removal from storage were caused by *Phytophthora*, *Pezizella*, *Rhizoctonia* sp. and *Botrytis*, with rot caused by *Phytophthora* greatly exceeding the other three in 11 of the Klondike lots and about equaling them in the other Klondike lot and two lots of Aroma. At temperatures between 40 and 45°F., all of the fungi named, but especially *Phytophthora*, were able to grow so rapidly that within four days they rendered a large part of the fruit worthless. Most of the infection was undoubtedly present though not apparent externally when the fruit was stored. The remainder must have developed through spread of *Phytophthora* from diseased to healthy berries. Evidence that this had happened was seen repeatedly during the making of counts, and later during the inspection of carload lots of strawberries on the Chicago market. The problem, however, is one which cannot be solved without further investigation.

#### CONTROL EXPERIMENTS

Strawberries used in the holding tests discussed above came from a number of fields near Beebe in which experiments had been conducted on control of fruit rots by spraying and dusting. The details of these experiments and the apparent effect of spraying and dusting on percentage of total rot have been reported (13). The data on leather rot are given in Table X, together with data for "other rots" and total rot, which are included for purposes of comparison.

TABLE X.—*Effect of spraying and of dusting on fruit rots of strawberries, at Beebe, Ark., 1923*

	Holding tests, per cent of rot four days after picking			
	Spray	Copper dust	Sulphur dust	Control.
<b>KLONDIKE-THORNTON PLOTS</b>				
Leather rot.....	22.5	24.3	0	34.8
Other rots.....	2.1	1.7	0	1.8
Total rot.....	24.6	26.0	0	36.6
<b>KLONDIKE-ABINGTON PLOTS</b>				
Leather rot.....	16.3	16.8	0	18.0
Other rots.....	2.8	5.8	0	10.3
Total rot.....	19.1	22.6	0	28.3
<b>AROMA-THORNTON PLOTS</b>				
Leather rot.....	17.2	19.6	4.5	17.7
Other rots.....	21.9	40.4	37.0	42.9
Total rot.....	39.1	55.8	41.6	60.3

The results presented above indicate some control of leather rot, though not such as would be considered satisfactory from a commercial point of view. It should be remembered, however, that they were obtained during an extremely wet season, in fields known to have suffered heavily from rot the previous year. It is planned to repeat these experiments another year.

It is planned also to test the value of mulching as a means of preventing rot. Mulching is known to be good practice in other strawberry sections, and it proved to be quite effective during the season of 1923, in one field near Beebe, where it was tried out in a small way by a grower. The material used was pine needles, applied in such a way that when well settled they made a layer about an inch thick. The writer visited this field on several occasions, but failed each time to find rot of any kind in the mulched rows; in the unmulched rows alongside, leather rot and other rots were fully as common as in any of the unmulched fields in the Beebe district. No counts were made.

The scarcity of leather rot in the district around Hammond, La., has already been referred to; attention has also been called to the fact that this district has as heavy rainfall as the strawberry districts farther north. But in the Hammond district practically all strawberry fields are mulched. In the writer's opinion this is precisely the reason why the disease is so scarce there. It has been found, but only in a few unmulched or poorly mulched fields.

### SUMMARY

1. A serious disease of strawberry fruits is reported which is apparently new to American phytopathological literature, though what seems to be the same disease was reported by Osterwalder (5) in 1912 as occurring in Switzerland.

2. It is demonstrated that the fungus, a *Phytophthora*, constantly associated with this disease is pathogenic to strawberry fruits. Evidence is presented which indicates that this *Phytophthora* is identical with *P. cactorum*, first described by Lebert and Cohn and reported in the United States as parasitic on apples, pears, ginseng and rhubarb.

3. The disease is described in detail and the common name of "leather rot" is suggested for it.

4. It is demonstrated by this investigation that there is a close relation between leather rot and rainfall, and that in the region studied, the rot can be expected to reach a maximum within three to four days after heavy rains. Temperature is important also but its effect can not be determined without further study.

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PLATE 1

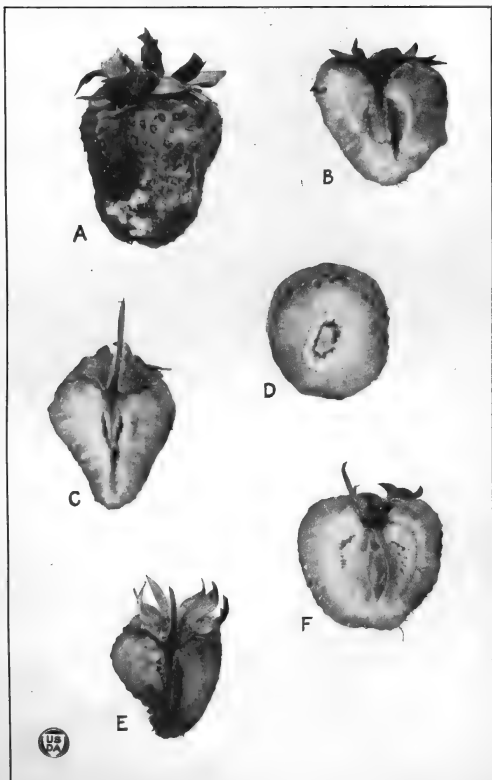
Leather rot of strawberry caused by *Phytophthora cactorum* (Leb. et Cohn) Schroeter.

A.—External appearance of rotting strawberry showing patches of the white mycelium of the fungus.

B, C.—Sections of berries showing the “water-soak” condition.

D, E, F.—Sections showing characteristic browning of the vascular tissue.





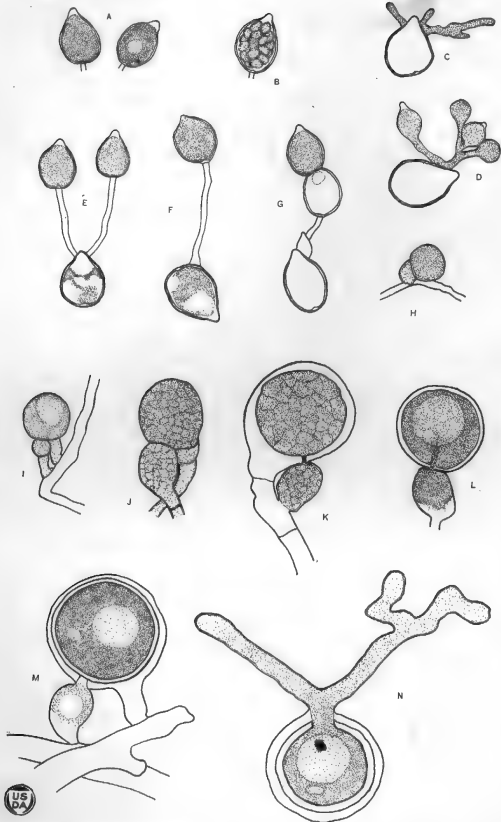


PLATE 2

Leather rot of strawberry caused by *Photophthora cactorum* (Leb. et Cohn) Schroeter.

A, B.—Conidia.

C to G.—Secondary and tertiary conidia.

H to M.—*Paragunous antheridia*.

N.—Germinating oospore.

# THE CAMBIUM CURCULIO, CONOTRACHELUS ANAGLYPTICUS SAY <sup>1</sup>

By FRED E. BROOKS, *Entomologist, Fruit Insect Investigations, Bureau of Entomology*, with detailed description of larva and pupa by R. T. COTTON, *Entomologist, Stored-Product Insect Investigations, Bureau of Entomology, United States Department of Agriculture*

## INTRODUCTION

Orchardists in the eastern part of the United States who practice jarring plum and peach trees to capture the beetles of the plum curculio, *Conotrachelus nenuphar* Herbst, occasionally obtain with these beetles specimens of the nearly related species *Conotrachelus anaglypticus* Say. This beetle is slightly smaller and somewhat more active than the plum curculio but in a general way resembles it rather closely, although there is little difficulty in distinguishing between the two. The larva is found in a variety of situations, but a frequent place of feeding and development is in the cambium around wounds in the bark of various kinds of orchard and forest trees. On account of this feeding habit the common name "cambium curculio" is here applied to the insect.

In the year 1910 E. L. Jenne, working under the direction of Dr. A. L. Quaintance, in charge of Fruit Insect Investigations in the Bureau of Entomology, made careful observations on the egg-laying habits and larval development of this insect at Gainesville, Ga. Mr. Jenne observed the species as a possible enemy of the fruit of the peach. In 1922 Oliver I. Snapp, also of the office of Fruit Insect Investigations, made observations on the insect as an enemy of peaches, at Fort Valley, Ga. Data obtained by both Jenne and Snapp are included in this paper, together with those collected by the writer in West Virginia over a period of several years. The studies in West Virginia have revealed the insect as an habitual feeder under the bark of fruit and other trees.

Both Jenne and Snapp found the larvae of the cambium curculio feeding in peaches in company with the larvae of the plum curculio. This association in feeding of the two similar species makes it important that observers be able to distinguish between them while in the larval stage. Accordingly, R. T. Cotton, a specialist in curculionid larvae, who is employed under the direction of Dr. E. A. Back, Stored-Product Insect Investigations in the Bureau of Entomology, furnished upon request the drawings (Pls. 2, 3, and 4) and comparative descriptions that are used in this paper.

## HISTORY AND DISTRIBUTION

This insect was named and described in 1831 by Thomas Say (7, p. 18).<sup>2</sup> Since the publication of the original description the species has been taken under a variety of conditions and in many localities. LeConte and Horn (6, p. 234), writing in 1876, record it from Massachusetts, Georgia, Kansas, and Texas. Blatchley and Leng (1, p. 481) add to its range the States of New Jersey, Michigan, Iowa, Illinois, Indiana, and Florida. The last named authors state that it is "abundant from New England to Michigan and Iowa, south to Florida and

<sup>1</sup> Received for publication April 22, 1924.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 386.

Texas." Scott and Fiske (8, p. 34) took it commonly in jarring peach and plum trees at Fort Valley, Ga. An unpublished note in the Bureau of Entomology shows that it was collected by a correspondent at Mount Solon, Va., in 1899. Felt (3, p. 544) records it as occurring at Poughkeepsie, N. Y., and in the Adirondack Mountains of the same State. It is recorded from the District of Columbia by Ulke (9, p. 34, 55), from southwestern Pennsylvania by Hamilton (4, p. 376), and from the vicinity of Cincinnati, Ohio, by Dury (2). In 1910 Jenne (5) collected it in numbers from peach trees at Gainesville, Ga., and in 1922 Snapp jarred numerous specimens from peach trees at Fort Valley, Ga. Data furnished by Thomas H. Jones, at the time entomologist of the Louisiana Agricultural Experiment Station, Baton Rouge, La., show that he reared adults of this species from larvae found at New Roads and Baker, La. The present writer has found the species abundant at French Creek, W. Va., and in other localities of that State. It would seem from the foregoing that the insect is distributed rather generally throughout much of the eastern and central portions of the United States.

### FOOD HABITS

Very few references to the feeding habits of this species are published. Say (7, p. 18) says of it "Breeds in the fruit of hickory (*Juglans*)."

Blatchley and Leng (1, p. 480) record taking the beetles from asters in Indiana and under moist bark and on various bushes in New Jersey. Felt (3, p. 544) states that beetles were taken on two successive days on a slippery elm tree that had been stripped of bark. This, he considered, indicated a certain attraction for this food plant.

Jenne, in his unpublished notes, speaks as follows of seven beetles that were jarred from peach trees at Gainesville, Ga.:

The beetles were kept in a jelly glass with fresh peach leaves and fruit. No feeding was done on either fruit or foliage, except that they punctured the fruit at the receptacle of the stem. When the fruit was cut open the beetles would feed freely on it, but they would not puncture the skin.

In speaking of the larvae Jenne says, "In no case were they able to enter peaches except through a previously made break in the skin." From the 7 beetles referred to above Jenne reared 79 adults which "also refused to puncture the skin of either peaches or apples, but fed freely on these fruits when cut open."

In 1922 Snapp, while working with peach insects at Fort Valley, Ga., found that the beetles would oviposit in sound peaches. His unpublished notes state that 10 beetles were confined in a battery jar and supplied with peaches free from feeding or egg punctures. "The peaches were removed after they had been exposed to the beetles 48 hours and placed in wire cylinders. A number of eggs were deposited." The larvae from these eggs matured in the fruit and were permitted to enter the soil. Later a number of adults came through. With reference to these observations Snapp states, "The peach fruits exposed to the adults 48 hours, and in which eggs were deposited, were all sound peaches properly matured to that period in their development. Only peaches free from signs of any egg laying or feeding punctures were used for the purpose." He adds further, "I am of the opinion that each season a small proportion of the wormy peaches in Georgia is due to *anaglypticus*. Heretofore all Georgia wormy peaches have been attributed to the work of *nenuphar*."

Theo. Pergande, of the Bureau of Entomology, entered the following unpublished note in the bureau files: "May 24, 1899. Received from Jas. T. Clark, Mount Solon, Va., one specimen of this beetle (*Conotrachelus anaglypticus*), which he found, with several others which escaped him, in black knot on his plum trees, in which they had evidently been breeding."

April 27, 1923, Thos. H. Jones furnished the writer with data which show that he had reared beetles of this species from cotton bolls collected in two localities

in Louisiana. According to Mr. Jones the larvae in feeding in the bolls seem to prefer to work around the stem ends. He stated that the larvae were fairly common in the bolls but that he was unable to decide whether they cause primary injury or follow other injury.

The writer has made repeated unsuccessful attempts to rear larvae of the cambium curculio in sound peaches and apples. In these experiments the beetles deposited their eggs freely in the fuzz on the surface of peaches but the larvae on hatching did not enter the fruit. When the skin and flesh of peaches and apples were cut, however, the beetles oviposited in the wounds and the larvae developed successfully in the fruit.

Observations made over a period of several years in West Virginia, where this insect is abundant, indicate that the normal place for oviposition and larval development is around the borders of fresh wounds in the bark of many kinds of orchard and forest trees. The writer has captured beetles and found the larvae feeding at bark wounds in the following species of trees: Apple, *Malus sylvestris*; pear, *Pyrus communis*; pignut, *Hicoria glabra*; American hornbeam, *Carpinus caroliniana*; sweet birch, *Betula lenta*; American beech, *Fagus grandifolia*; American chestnut, *Castanea dentata*; white oak, *Quercus alba*; chestnut oak, *Q. montana*; red oak, *Q. borealis maxima*; tulip tree, *Liriodendron tulipifera*; service berry, *Amelanchier canadensis*; red maple, *Acer rubrum*; tupelo, *Nyssa sylvatica*; flowering dogwood, *Cornus florida*; and sourwood, *Oxydendrum arboreum*. The bark of many of these trees was scarified for the purpose of attracting the insects. Both larvae and beetles, however, were observed and collected under other conditions. They were found in great numbers in stumps and ends of logs cut in the forest for lumbering purposes, feeding about wounds in apple bark made by falling hailstones, and feeding in apple bark at edge of wounds made in pruning; larvae were found in wounds in dogwood bark made by boring larvae of *Aegeria* sp., in ax wounds made by lumbermen in trunks of tulip and dogwood trees, and in the edge of wounds in sweet birch made by boys in peeling bark for camp shelter; beetles were found hiding in fresh perforations made in pear bark by the yellow-bellied sapsucker, *Sphyrapicus varius varius*; were jarred from trees of peach, plum, and black walnut, *Juglans nigra*, and were found hiding in curled leaves on a hickory tree.

It is not unusual to find in June and July as many as a score of larvae feeding around a single wound in the bark of a tree trunk. In one case 24 larvae were taken from the edge of a wound in apple bark and in another 38 larvae were found under a small piece of bark at a wound in chestnut. Very little preference is shown for particular species of trees, although apple and pear seem to be especially attractive. Beetles, however, were attracted to every trapping place made in June, regardless of the species of tree used, and later the larvae were always present.

#### NATURE OF INJURY

Observations have shown that the cambium curculio is injurious in two distinct ways. It attacks the fruit of the peach and enlarges and prevents the healing of wounds in the bark of various kinds of trees (Pl. 1, C). While neither Jenne nor the writer found cases of injury to sound peaches, Snapp discovered that a small proportion of wormy peaches in Georgia is apparently due to this insect. There is also a possibility that sound cotton bolls are attacked and injured by the larvae, as has been pointed out by Jones. The larvae, in feeding in the bark, mine inward through the cambium from the edges of fresh wounds (Pl. 1, C). They do not mine extensively, but their feeding area, which is usually in the form of a band around the wound, varies from half an inch to 2 inches in width. Throughout this band all the cambium is likely to be destroyed and the wound enlarged to that extent. As the wound grows old the larvae cease to attack.

## THE EGG

## DESCRIPTION

The egg (Pl. 1, D) of the cambium curculio is somewhat variable in size and shape. Average specimens are oval, 0.7 mm. long by 0.4 to 0.45 mm. wide. The color is translucent white, with a yellowish tinge, the surface is slightly wrinkled, and the contents of the egg are plainly visible through the shell.

## OVIPOSITION

Jenne states that eggs on peaches are deposited in the fuzz, the skin never being punctured. The writer observed that caged beetles would oviposit in the fuzz of peaches and on the downy surface of young apple shoots, but no eggs were found on the smooth skin of apple fruits nor on smooth apple bark. When fresh twigs were placed in the cages with slits cut in the bark the beetles would oviposit in the slits in preference to any other location. Whenever eggs were placed on a fuzzy surface the beetles would draw a little of the available fiber around the eggs. Many eggs were found partially embedded in the moist fiber of the inner bark around fresh wounds in trees. Jenne states that of 88 eggs observed all were laid in the night and 41 hatched in the night.

## INCUBATION

At Gainesville, Ga., Jenne made observations on the incubation period of 188 eggs, the results of which are set forth in Table I.

TABLE I.—*Incubation period of 188 eggs of the cambium curculio at Gainesville, Ga., in 1910*

Number of eggs hatching	Duration of period
	<i>Days</i>
22	2
58	2.5
67	3
27	3.5
13	4
1	4.5

The average time required for hatching was 2.87 days. The first of the lot of 188 eggs was laid on May 20 and the last on July 10, oviposition therefore extending over a period of 52 days. Eggs laid early in the season were slightly slower in hatching than those laid late in the season. The 24 eggs laid in May required on an average 3.5 days to hatch, while the 13 laid in July required 2.79 days to hatch. The increasing warmth of the advancing season probably accounts for the shortening of the incubation period. Numerous eggs laid in West Virginia hatched in from 3 to 4 days. Jenne kept an egg record of seven beetles, but he does not state whether they were all females. The lot produced 188 eggs, an average of 26.86 each if they were all females.

## THE LARVA

DESCRIPTION <sup>3</sup>

Mature larva of *Conotrachelus anaglypticus* (Pls. 2 and 3) from 7 to 9 mm. in length and from 1.5 to 2 mm. in width. Body white, fleshy, and legless, almost cylindrical but somewhat flattened ventrally. Ten abdominal segments, the ninth reduced and flattened, the tenth small and ventral.

<sup>3</sup> By R. T. Cotton.

Head light yellowish-brown, the anterior margin and mandibles much darker. Head, with labium and clypeus included, subquadrate, the width but slightly greater than the length. Width of head about 1.06 mm. Epicranial and frontal sutures distinct.

Frons subtriangular, with a dark median line indicating the carina extending about two-thirds of the distance from the posterior angle to the anterior margin of frons. Near anterior margin of frons on each side a group of two setae with a sensory spot between. Setae and sensory spot arranged in a straight, transverse line. In posterior angle of frons on each side two setae with sensory spot between arranged in a longitudinal curved line.

Clypeus broader than long, sides narrowing sharply to apical angles. Epistomal suture with two fine setae and an intermediary sensory spot on each side. Labrum subtriangular, broader than long, with three pairs of dorsal and six pairs of marginal setae.

Each epicranial half with thirteen setae and three sensory spots arranged as shown in Plate 2, H. One inconspicuous ocellus on each side. Antenna small, fleshy, two-jointed; basal joint with several small papillae.

Mandibles stout, subtriangular, slightly bifid at tip, excavated along the irregularly serrated cutting edge, no molar part, dorsal area with two setae.

Maxillary mala entire, tip obtuse, dorsal and ventral surfaces smooth. Dorsal surface with a longitudinal row of eight unbranched, stout setae. Ventral surface with four setae and a sensory spot near tip. Maxillary palp extending slightly beyond mala, two-jointed, borne by large membranous palpifer; proximal joint thick, cylindrical, bearing two sensory spots and with a small seta on the apical membrane; distal joint fingerlike, bearing several small terminal papillae. Three other large setae on maxilla, two of them near base of palpifer and the other about midway between palpus and end of cardo. A very minute seta with a sensory spot near stipes labii but usually concealed by the folded skin.

Mentum, submentum, and maxillary articulating area fused into a fleshy lobe. Three pairs of setae. Eulabium posteriorly enforced by a medium, triangularly bent chitinization. Between the palpi a small lobelike ligula. Labial palp short, conical, two-jointed; distal joint with several small terminal papillae. Eulabium bearing two small setae and two sensory spots on ventral surface. Ligula with two pairs of small setae and two sensory spots. Floor of buccal cavity composed of a fleshy hypopharynx laterally somewhat spinose and strengthened with small lateral chitinizations (not shown in figure). Hypopharyngeal bracon whitish and band-shaped, loosely connected with thin chitinizations laterally on hypopharynx.

Epipharynx carrying a pair of epipharyngeal rods. Between the rods two pairs of small, stout setae and a pair of three-lobed sensory spots. Prothorax dorsally not divided, thoracic plate lightly chitinized and provided with eight pairs of setae and two pairs of sensory spots. First thoracic spiracle pushed into prothorax but located rather far from anterior prothoracic margin; elongate, bifore, with the fingerlike air tubes pointing dorsad; at least twice as large as abdominal spiracles. Mesothoracic and metathoracic segments above divided into a spindle-shaped praescutum, a scuto-scutellum, and an alar area. Praescutum with one pair of setae, scuto-scutellum with four pairs, and each alar area with one seta.

Sternum of thorax consisting of the eusternum with the presternum depressed in front of it, a coxal lobe on each side, the sternellum, and the poststernellum. Eusternum of each thoracic segment bearing a pair of setae.

First seven abdominal segments divided into praescutum, scutum, scutellum, postscutellum, and alar area. Praescutum bearing a pair of setae, each scutellum five setae, and each alar area one pair of setae. Below the tergal areas is the epipleurum with two setae, the hypopleurum with two setae, the coxal lobe with one seta, and the eusternum with two pairs of setae. Eighth abdominal



segment dorsally with only three setae on the poorly defined scutellum. Ninth abdominal segment somewhat flattened dorsally and terminated with two pairs of long, stiff setae.

Spiracles of first eight abdominal segments bifore, the fingerlike air tubes pointing caudad.

#### COMPARISON OF LARVAE OF CONOTRACHELUS ANAGLYPTICUS AND C. NENUPHAR <sup>4</sup>

The larvae of *Conotrachelus anaglypticus* and *C. nenuphar* (Pls. 2 and 3) are, like all species of this genus, very similar in form and appearance. The full-grown larva of *C. anaglypticus* is, however, somewhat shorter and noticeably less robust than that of *C. nenuphar*. The following measurements indicate the relative difference in size between the average full-grown larvae of the two species:

*C. anaglypticus*: Length 7 to 9 mm.; width 1.5 to 2 mm.; dorso-ventral thickness 1.2 to 1.5 mm.

*C. nenuphar*: Length 8 to 10 mm.; width 2 to 2.5 mm.; dorso-ventral thickness 2 mm.

The larval head of *C. anaglypticus* is much flatter dorso-ventrally than that of *C. nenuphar* and the sides of the head are not so rounded as in *C. nenuphar*.

The most noticeable difference between the two species is in the frons. In *C. nenuphar* the dark median line or carina extends from the posterior angle to about the middle of the frons, whereas in *C. anaglypticus* the carina is much longer and extends fully two-thirds of the distance from the posterior angle to the anterior margin of the frons. Near the anterior margin of the frons there are on each side a group of two setae and a sensory spot. In *C. nenuphar* the setae and sensory spot of each group are arranged in the form of a triangle, but in *C. anaglypticus* the setae and sensory spot are arranged in a straight line.

#### HABITS AND LENGTH OF LARVAL LIFE

The white legless larva (Pl. 1, C, E, and F) of the cambium curculio is rather active and has a habit of throwing itself when disturbed, by straightening with a snap from a curved position. Jenne says, "Full-grown larvae have a habit of leaping when irritated. They do this by curling their body up so as to hook the anal segment under the head. The body is then snapped out straight, causing the larva to jump from 3 to 4 inches along a smooth surface, or to a height of about 1 inch." The writer noticed that when a piece of bark under which the larvae were feeding was removed the insects would one by one throw themselves out of sight in the way described.

Jenne reared 88 larvae in fallen peaches, plums, and apples, most of the number being reared from peaches. The duration of larval life in the fruit of these 88 larvae is shown in Table II.

TABLE II.—Length of larval life of the cambium curculio in peaches at Gainesville, Ga., in 1910

Period in fruit	Number of larvae	Period in fruit	Number of larvae	Period in fruit	Number of larvae
<i>Days</i>		<i>Days</i>		<i>Days</i>	
11	1	19	7	25	4
13	1	20	10	26	2
14	10	21	8	28	1
15	5	22	3	29	2
16	5	23	4	30	4
17	6	24	3	33	1
18	11				

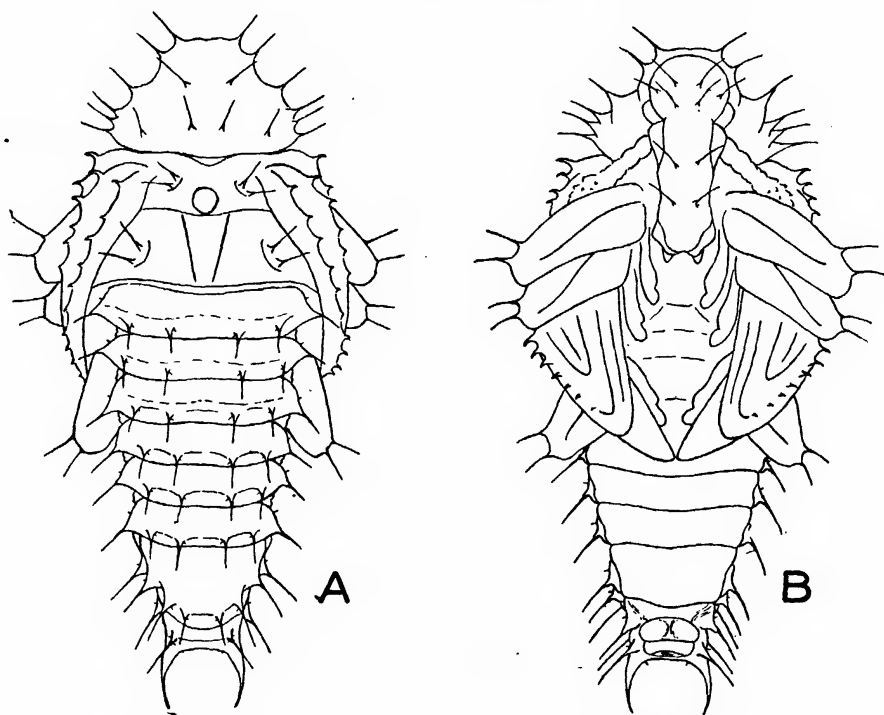
<sup>4</sup> By R. T. Cotton.

The insect in its active stages seems somewhat nocturnal in habits. Not only does oviposition take place principally at night, but 72 of the 88 larvae left their feeding places to enter the ground for pupation at night. The larvae burrow into the soil to a distance of from 1 to 3 inches to pupate; usually, however, pupation takes place about 1 inch beneath the surface of the soil.

## THE PUPA

### DESCRIPTION

Pupa of *Conotrachelus anaglypticus* (fig. 1) uniformly white in color; length about 5 to 5.5 mm. Head rounded, beak elongate and attaining base of mesothoracic legs; head with three pairs of setae borne on tubercles; beak with two pairs of setae borne on tubercles, one pair near base of antennae, the other pair between base of antennae and tip of beak. Prothorax subtriangular, rounded in front, with two pairs of antero-marginal setigerous tubercles, four pairs of latero-



Text fig. 1, A

Text fig. 1, B.

FIG. 1.—*Conotrachelus anaglypticus*: A, Pupa, dorsal view; B, same, ventral view.

marginal, and three pairs of dorsal, setigerous tubercles. Mesonotum and metanotum each provided with two rather closely placed setigerous tubercles on each side of median area.

Abdomen with eight distinct tergites, median area of each tergite with a transverse row of four setigerous tubercles each of which has at base a minute seta; lateral areas of each tergite with a setigerous tubercle at base of which is a small seta. Ninth abdominal segment ventral, armed with a pair of stout, terminal spines.

Tips of wing pads attaining the fifth abdominal sternite; tips of metathoracic tarsi not extending to tips of wing pads. Elytra armed with longitudinal rows of short hooks or spines.

The pupa of *C. nenuphar* closely resembles that of *C. anaglypticus* but may be readily distinguished by its much larger size. Specimens forwarded to the writer vary in length from 6.5 to 8 mm.

## LOCATION OF PUPA AND LENGTH OF UNDERGROUND PERIOD

The pupa (Pl. 1, F, and Pl. 4) occupies a smooth-walled cell within a spherical pellet of earth an inch or two below the surface of the ground. Jenne observed the time elapsing between the entering of the soil by the larvae and the emergence of the adults in 80 cases. Table III sets forth his data.

TABLE III.—*Number of days spent underground in the act of pupation by the cambium curculio at Gainesville, Ga., in 1910*

Number of insects	Period underground
	<i>Days</i>
1	17
13	18
13	19
25	20
18	21
6	22
1	23
2	24
1	29

Observations in West Virginia showed that a considerably longer period may be required in some cases. In one instance a number of larvae entered the soil under outdoor conditions on July 16. On September 15, 62 days later, live adults were still within the soil, although evidently ready to issue.

## THE ADULT

## DESCRIPTION

The adult cambium curculio (Pl. 1, A, B) is a small active snout-beetle from 3.5 to 5 mm. in length. The head, thorax, and under parts are dark brown to black and the legs and elytra reddish brown. The upper parts are clothed thinly with lighter colored hairs which form two narrow whitish lines on each side of the thorax, a broad, yellowish, oblique stripe at the base of each elytron, and a broad grayish band behind the middle of the elytra. The legs are marked, especially on the femora, with whitish bands of short hairs. The slightly curved snout is nearly as long as the head and thorax combined.

## SEASONAL OCCURRENCE AND LONGEVITY

The beetles make their appearance from hibernation fairly early in the spring. Blatchley and Leng (1, p. 481) record taking specimens at Dunedin, Fla., on February 27 and March 17. Jenne captured specimens at Gainesville, Ga., April 16–30. Snapp began to secure beetles of this species in jarring peach trees at Fort Valley, Ga., on April 17. At French Creek, W. Va., the beetles appear by the middle of May and are present throughout the summer, activity being greatest during June and July. Two of the beetles which Jenne collected in April at Gainesville, Ga., were still alive on the 5th of the following November, another lived until September 11, and another until October 15. Jenne obtained beetles of the second generation from July 8 to August 9. Sixty-one of these young beetles were placed in an outdoor cage containing dry oak leaves on November 4 for the purpose of observing winter mortality. Of the lot placed in the cage at least 59 per cent were still alive on March 8 of the following spring.

## LIFE CYCLE AND NUMBER OF GENERATIONS

Snapp found that in Georgia there are two generations of beetles annually; in West Virginia, on the other hand, there is but one.

Jenne recorded for 78 individuals the entire life cycle from the deposition of the eggs to the emergence of the adults from the ground. These records are given in Table IV.

TABLE IV.—Time required by the cambium curculio for completing its life cycle at Gainesville, Ga., 1910

Number of days	Number of insects	Number of days	Number of insects	Number of days	Number of insects
35	1	43	9	51	1
36	1	44	5	52	1
37	3	45	4	53	1
38	6	46	3	56	1
39	10	47	3		
40	3	48	5		
41	10	49	4		
42	7				

## NATURAL ENEMIES

At French Creek, W. Va., the adult of a small hymenopterous parasite was found hovering around a larva of the cambium curculio under the bark of a tree. It was believed to be ovipositing in the body of the curculio larva. The specimen was captured and determined later by R. A. Cushman of the Bureau of Entomology as *Thersilochus conotracheli* Riley. Thomas H. Jones reports that he has reared from cotton bolls infested by larvae of this curculio from New Roads, La., specimens of a tachinid fly determined by Dr. J. M. Aldrich as *Myiophasia globosa* Townsend. Since both the species mentioned are known to parasitize *Conotrachelus* larvae, it is probable that they were attacking the larvae of *anaglypticus*.

## METHODS OF CONTROL

Where this curculio attacks peaches there is little doubt that it will yield to jarring, spraying, and other control methods used against the plum curculio. In attacking the fruit the habits of the two species are so similar that separate treatments will not be called for. As a precaution against enlargement of bark wounds in fruit and other valuable trees by the larvae, the edges of fresh wounds made in the bark should be pared smooth and a heavy coat of white-lead paint, or some reliable tree paint, applied to all the injured parts. Such a coat of paint has been found effective in preventing the beetles from laying their eggs about the wounds.

## SUMMARY

*Conotrachelus anaglypticus* is a rather common species from Massachusetts to Florida and its range extends as far west as Iowa, Kansas, and Texas.

The species feeds in a great variety of situations. The beetles frequently occur on peach trees in company with those of the plum curculio, and the larvae have been found attacking peaches, cotton bolls, and the cambium and inner bark of many kinds of fruit, shade, and forest trees. In attacking the cambium of trees they work around the edges of wounds, retarding the healing process and enlarging the injured areas.

The delicate white eggs are mostly laid at night in situations where the larvae are to feed. Eggs hatch in from 2 to 4 days and a single beetle lays on an average from 25 to 30 eggs. The larvae usually feed from 2 to 4 weeks and then enter the ground to pupate. In late summer and autumn the beetles issue from the soil and soon thereafter hibernate, probably in litter on the ground. In Georgia there are two generations of beetles each year, but from West Virginia northward there is but one generation annually.

The species is probably attacked by at least two parasites. In West Virginia a hymenopterous species, *Thersilochus conotracheli* Riley, apparently attacks the larvae and in Louisiana a dipterous parasite, *Myiophasia globosa* Townsend, was reared from cotton bolls infested by the cambium curculio.

Where this insect attacks peaches it will probably yield to jarring, spraying, and other methods used against the plum curculio. As a precaution against injury to the cambium of valuable trees, the edges of any wounds in the bark should be promptly pared smooth and the injured areas treated to a coat of white-lead paint or some reliable tree paint.

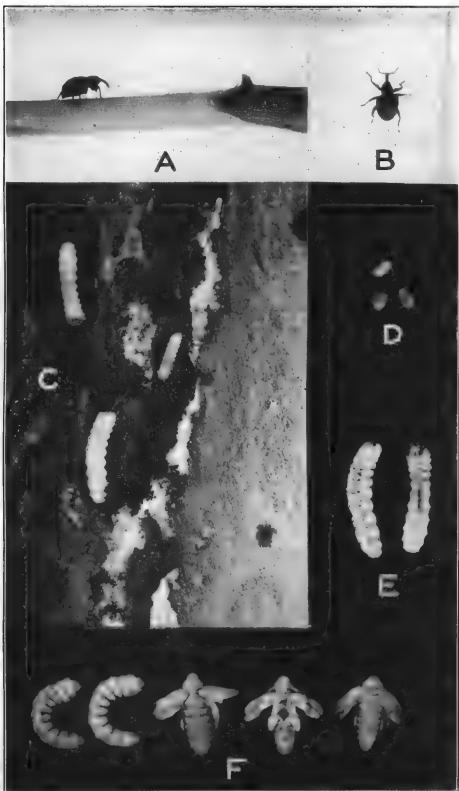
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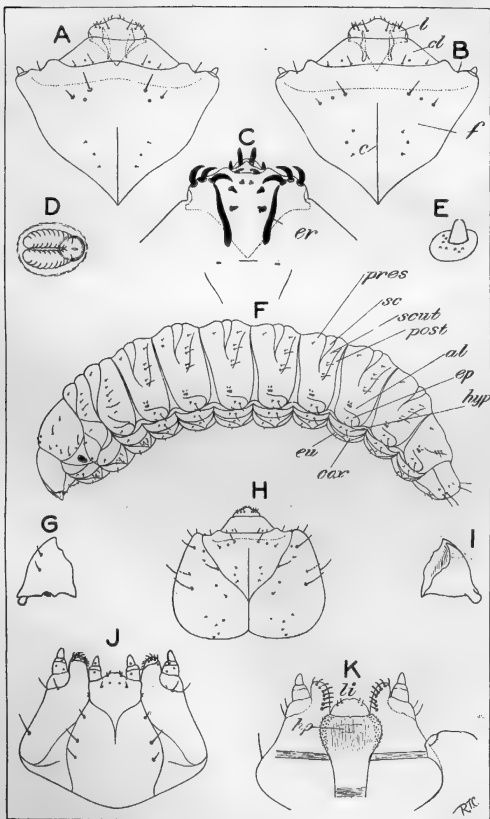
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PLATE 1

*Conotrachelus anaglypticus*

- A, B.—Adult curculios.  $\times 3$   
C.—Larvae mining into the live cambium from the dead wood on the right.  $\times 3$   
D.—Eggs.  $\times 4$   
E.—Larvae.  $\times 3$   
F.—Larvae (left two) and pupae (right three).  $\times 3$







## PLATE 2

*Conotrachelus nenuphar* and *C. anaglypticus*: Details of larvae.

A.—*Conotrachelus nenuphar*: Anterior portions of face.

B.—*C. anaglypticus*: Anterior portions of face. *c.* Carina; *cl.* clypeus; *f*, frons; *l*, labrum.

C.—*C. anaglypticus*: Epipharynx. *er*, Epipharyngeal rod.

D.—*C. anaglypticus*: Thoracic spiracle.

E.—*C. anaglypticus*: Antenna.

F.—*C. anaglypticus*: Side view of larva. *al*, Alar area; *cox*, coxal lobe; *ep.* epipleurum; *eu*, eusternum; *hyp*, hypopleurum; *post*, postscutellum; *pres*, prescutum; *sc*, scutum, *scut*, scutellum.

G.—*C. anaglypticus*: Mandible, dorsal surface.

H.—*C. anaglypticus*: Front view of face.

I.—*C. anaglypticus*: Mandible, ventral surface.

J.—*C. anaglypticus*: Ventral mouth parts.

K.—*C. anaglypticus*: Dorsal view of maxillae, ligula, and hypopharynx. *li*, Ligula; *hp*, hypopharynx.

### PLATE 3

*Conotrachelus nenuphar* and *C. anaglypticus*: Details of larvae.

A.—*Conotrachelus nenuphar*: Anterior portions of face.

B.—*C. anaglypticus*: Anterior portions of face. *ant*, Antenna; *c*, carina; *cl*, clypeus; *em*, epistoma; *f*, frons; *l*, labrum.

C.—*C. nenuphar*: Epipharynx. *er*, Epipharyngeal rod.

D.—*C. nenuphar*: Mandible, dorsal view.

E.—*C. nenuphar*: Mandible, ventral view.

F.—*C. nenuphar*: Side view of larva. *al*, Alar area; *cox*, coxal lobe; *ep*, epipleurum; *eu*, eusternum; *hyp*, hypopleurum; *pres*, prescutum; *post*, post-scutellum; *sc*, scutum; *scut*, scutellum.

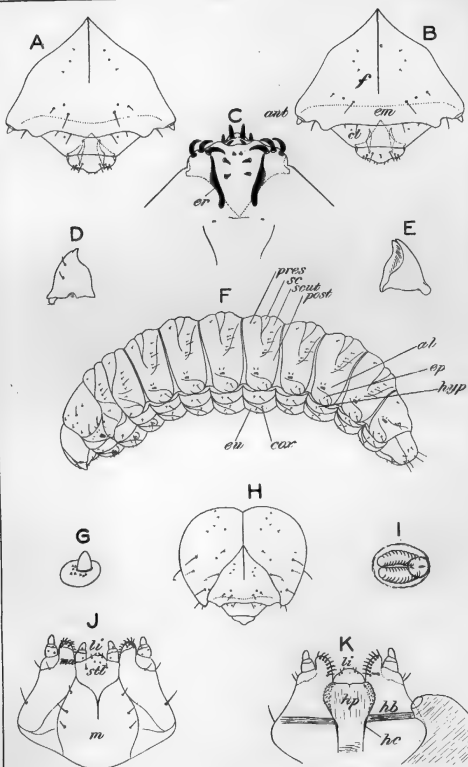
G.—*C. nenuphar*: Antenna.

H.—*C. nenuphar*: Front view of face.

I.—*C. nenuphar*: Thoracic spiracle.

J.—*C. nenuphar*: Ventral mouth parts.

K.—*C. nenuphar*: Dorsal surface of maxillae, ligula, and hypopharynx. *li*, Ligula, *hb*, hypopharyngeal bracon; *hc*, hypopharyngeal chitinization; *hp*, hypopharynx; *li*, ligula.



# RELATION OF THE MOLECULAR PROPORTIONS IN THE NUTRIENT SOLUTION TO THE GROWTH OF WHEAT<sup>1</sup>

By M. C. SEWELL

*Associate Professor of Soils, Department of Agronomy, Kansas Agricultural Experiment Station*

## INTRODUCTION

The investigation herein reported was made to determine the comparative effects of three salts in varying proportions upon the growth of wheat in separate stages, namely, the seedling phase, the vegetative phase, and the fruiting phase. The salts used were potassium di-hydrogen phosphate, calcium nitrate, and magnesium sulphate. The problem was attempted with the expectation that possibly the data obtained would give some knowledge on which to base fertilizer practice pertaining to the wheat crop.

The results seem to show an interesting relation between the nitrogen and phosphorus potassium salts in the growth response. With a high molecular proportion of nitrogen, a maximum grain yield was obtained with a low molecular proportion of the phosphorus potassium salt. With a low molecular proportion of nitrogen, equally high grain yields were obtained only with a high proportion of the phosphorus potassium salt.

## EXPERIMENTAL DATA

Wheat, first germinated in a nutrient solution, was grown in quartz sand, five plants to a culture. The containers were cylindrical half-gallon jars, glazed within and without, and holding 2,500 gm. of dry sand. After the seedlings were planted the jars were sealed with a wax preparation, and a paraffined paper cone was inserted as a supply orifice for adding water and the nutrient solutions. Surplus solution was removed by suction through a glass tube, plugged with glass wool at the lower end, which extended to the bottom of the jars. Measurements were made of transpiration losses, old solution removed, and new added every three and one-half days. During the fruiting phase distilled water was added daily between the three and one-half day periods.

The salts were used in 21 partial volume molecular proportions, all having a calculated osmotic value of approximately 1 atmosphere at 25° C. but differing (by increments of 1.8) in salt proportion.

The seedling phase consisted of a period of five weeks, the vegetative phase a period from the end of the seedling phase until the first appearance of flowering, and the reproductive phase extended from the vegetative phase until the maturity of the five best cultures of the group.

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<sup>1</sup> Accepted for publication Dec. 15, 1923. Department contribution No. 150. The investigation reported in this paper was conducted in cooperation with the special committee on salt requirements of representative agricultural plants, National Research Council, Division of Biology and Agriculture. Dr. H. H. King, Department of Chemistry, Kansas Station, assisted in the experiment during the seedling phase of growth.

Five control cultures were used during each phase of growth studied. The control solution was Shive's<sup>2</sup> best solution, with a molecular proportion of 3.77  $\text{KH}_2\text{PO}_4$ , 1.09  $\text{Ca}(\text{NO}_3)_2$ , and 3.14  $\text{MgSO}_4$ . This is a combination comparable to culture No. 16 in the accompanying figures.

In the study of the fruiting phase, cultures were begun January 2, 1923; the vegetative phase was marked as beginning February 6, and the fruiting phase, April 17. The plants were harvested June 1.

The daily maximum and minimum temperatures during these periods of growth are presented in Table I.

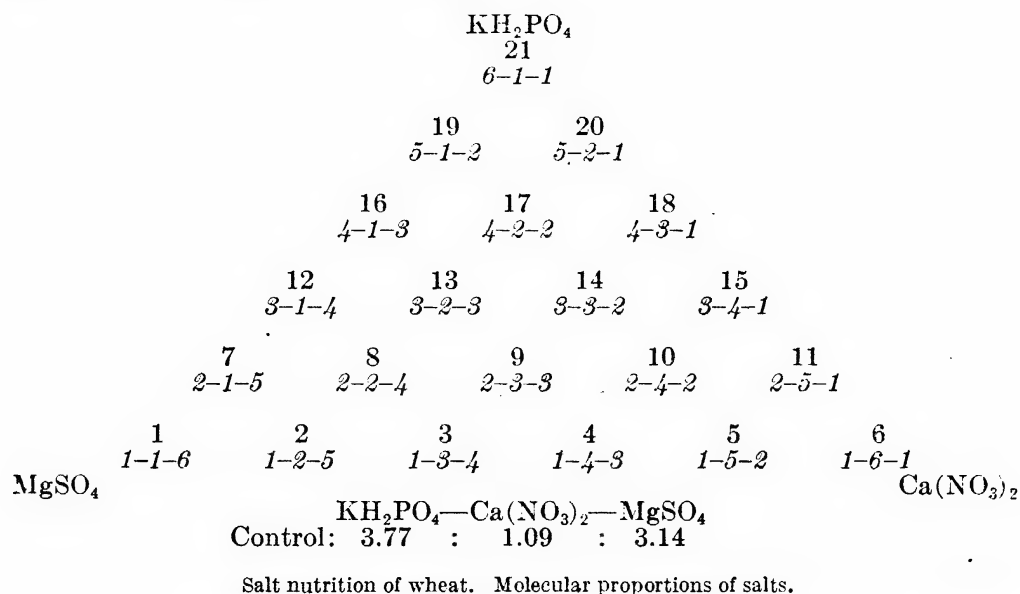
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<sup>2</sup> SHIVE, J. W. A STUDY OF PHYSIOLOGICAL BALANCE FOR BUCKWHEAT GROWN IN THREE-SALT SOLUTIONS. N. J. Agr. Exp. Sta. Bul. 319, 63 p., illus. 1917.

TABLE I.—Greenhouse temperatures averaged by five-day periods during the growth of wheat

Date, 1921, 1922	Seedling phase		Date, 1922	Seedling phase for vegetative study		Date, 1923	Seedling phase for fruiting study		Date, 1922	Vegetative phase		Date, 1923	Vegetative phase for fruiting study		Date, 1923	Fruiting phase	
	Max.	Min.		Max.	Min.		Max.	Min.		Max.	Min.		Max.	Min.		Max.	Min.
Dec. 6-10.....	° F. 82	° F. 59	Feb. 21-25.....	° F. 69	° F. 65	Jan. 1-5.....	° F. 72	° F. 55	Apr. 2-6.....	° F. 63	° F. 59	Feb. 10-14.....	° F. 76	° F. 65	Apr. 18-22.....	° F. 89	° F. 57
11-15.....	78	60	20-Mar. 2.....	64	59	6-10.....	74	59	7-11.....	64	61	15-19.....	79	63	23-27.....	94	60
16-20.....	82	56	Mar. 3-7.....	63	59	11-15.....	73	60	12-16.....	66	62	20-24.....	77	62	28-May 2.....	85	54
21-25.....	76	55	8-12.....	70	61	16-20.....	77	68	17-21.....	66	59	25-Mar. 1.....	80	67	May 3-7.....	87	57
26-30.....	75	65	13-17.....	65	62	21-25.....	73	62	22-26.....	66	59	Mar. 2-6.....	79	65	8-12.....	101	52
Jan. 1-5.....	75	56	18-22.....	70	63	26-30.....	72	63	27-May 1.....	69	62	Mar. 6-11.....	83	63	13-17.....	93	45
6-10.....	74	62	23-27.....	67	60	31-Feb. 4.....	72	63	May 2-6.....	68	63	17-21.....	76	60	18-22.....	93	52
			28-Apr. 1.....	69	63	Feb. 5-9.....	72	61	7-11.....	72	65	22-26.....	75	58	23-27.....	105	54
									12-17.....	68	64	27-31.....	80	63			
									18-21.....	68		Apr. 1-5.....	87	63			
												Apr. 6-10.....	90	61			
												11-16.....	90	60			

The 21 different molecular combinations of the three salts and the position of the cultures arranged in triangles are shown below. A duplicate triangle of cultures was conducted for each phase of growth. Since similar results were obtained in the duplicate cultures, the data from only one triangle for each phase of growth are presented here.



To visualize the data and to facilitate comparisons, figures 1, 2, 3, and 4 are given in lieu of tables. The sizes of the circles represent the comparative yields

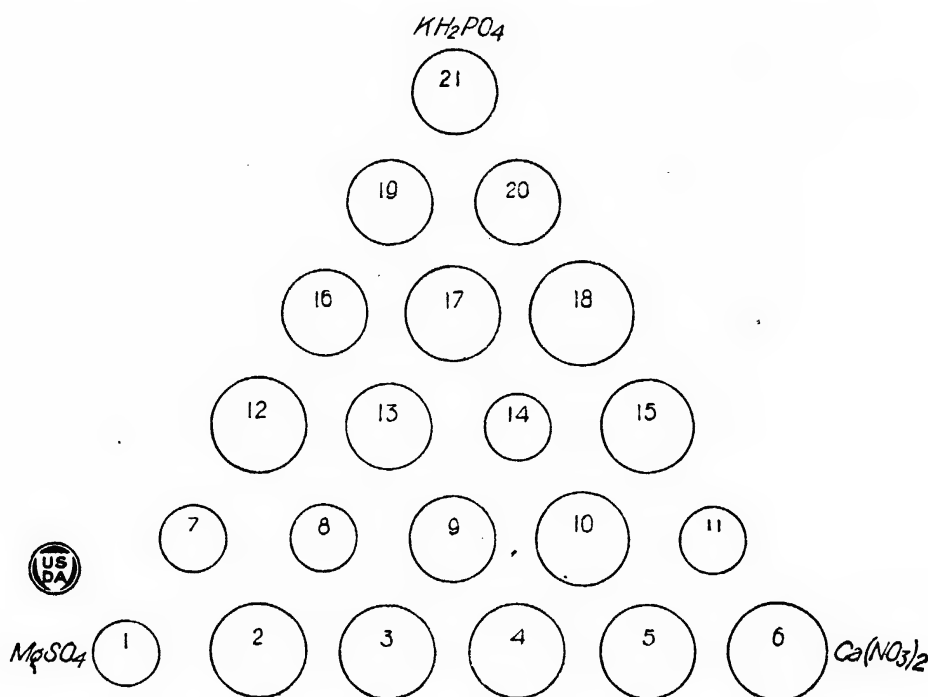


FIG. 1.—Salt nutrition of wheat: Seedling phase. Triangle 1, dry weight of tops.

measured in terms of dry weight of tops and, for the fruiting phase, dry weight of grain. The leaf colors of each of the various cultures during the seedling

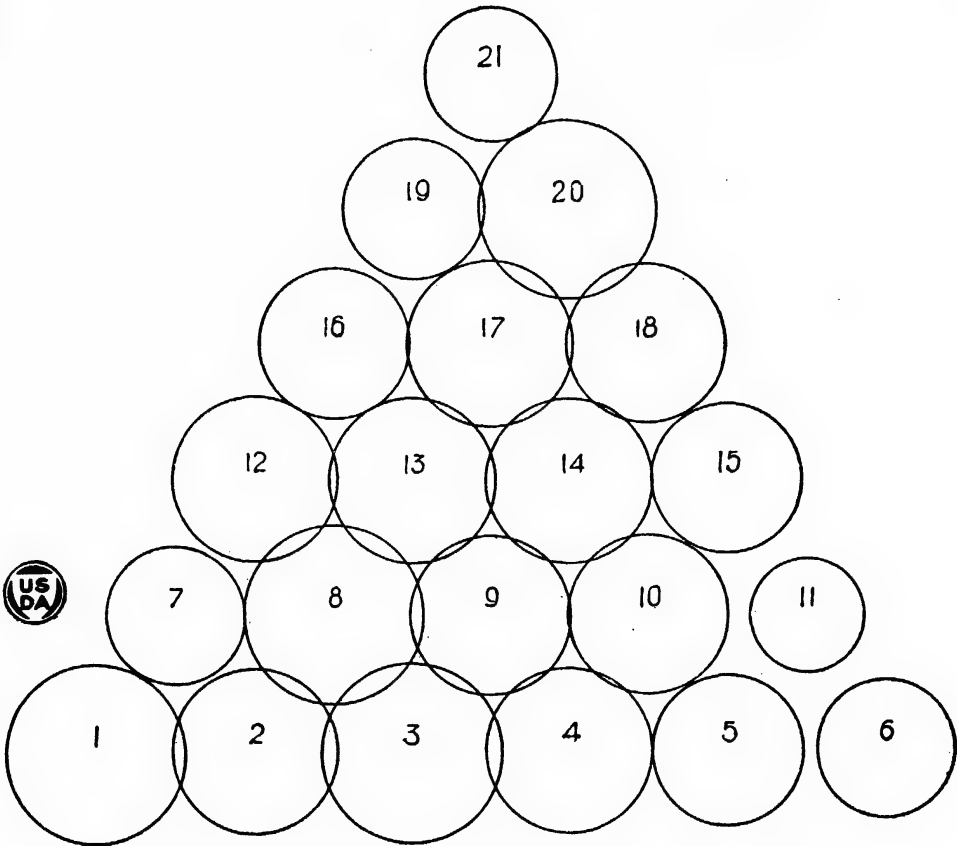


FIG. 2.—Salt nutrition of wheat: Vegetative phase. Triangle 1, dry weight of tops.

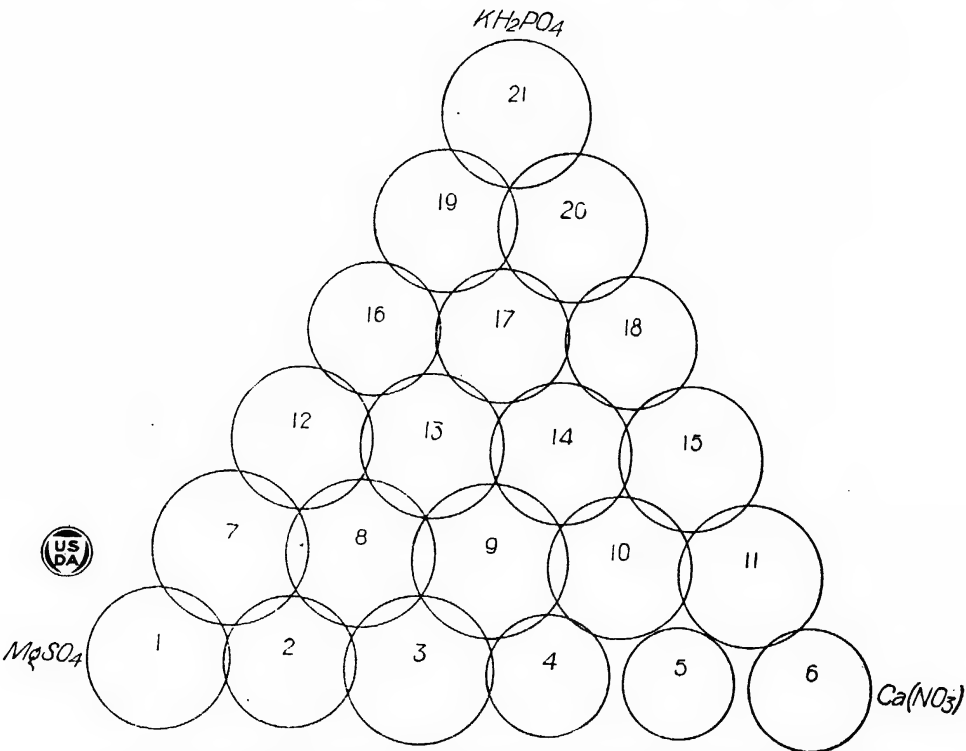


FIG. 3.—Salt nutrition of wheat: Fruiting phase. Triangle 1, dry weight of tops.



phase, identified by Ridgway's "Color Standards and Color Nomenclature,"<sup>3</sup> may be described as follows: 1, 2, 5, mineral green (*Pl. XVIII*); 3, 4, Veronese green (*Pl. XVIII*); 7, Tiber green (*Pl. XVIII*); 8, rivage (*Pl. XVIII*); 9, lumiere (*Pl. XVII*); 10, glass green (*Pl. XXXI*); 6, 11 to 21, deep turtle green (*Pl. XXXII*).

Figure 4, representing the fruiting phase and yield of ripe grain, shows the best solutions to be numbers 3, 10, and 19. The duplicate triangle for this same phase, of which results are not here given, showed the best solutions in the same outstanding way to be numbers 4, 9, and 20. These are adjoining cultures on the triangles, and are hence of nearly the same molecular proportions.

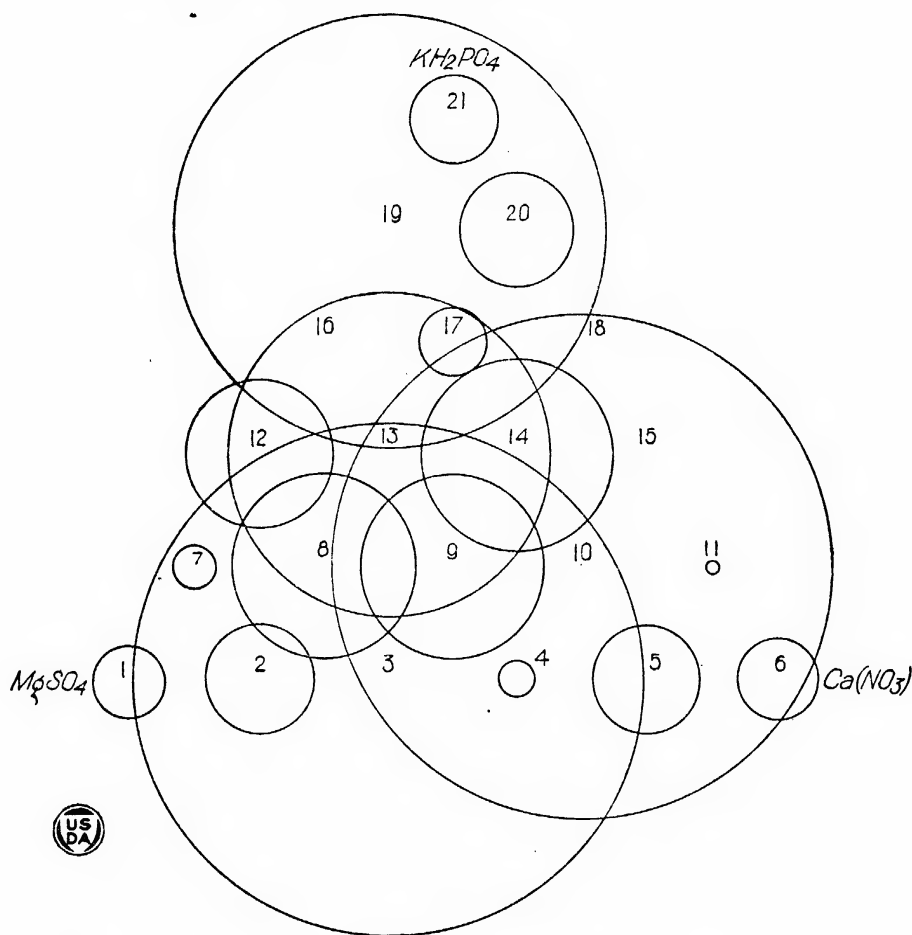


FIG. 4.—Salt nutrition of wheat: Fruiting phase. Triangle 1, weight of ripe grain.

An examination of figures 1, 2, and 3 shows that these solutions are favorable to growth during the seedling and vegetative phases, although solution No. 10 was off-color during the seedling stage.

The best solution for the seedling phase of growth was considered to be culture No. 17, and for the vegetative phase culture No. 20. The green weights of tops and weights of roots were also considered in selecting these particular solutions as best for the respective phases of growth.

To determine the best molecular proportion of these salts for the fruiting phase, all cultures were carried through the seedling phase with solution 17, and through the vegetative phase with solution 20. At the beginning of the fruiting phase,

<sup>3</sup> RIDGWAY, R., COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912.

the 21 different solutions were added to the designated cultures. In the order of  $\text{KH}_2\text{PO}_4$ ,  $\text{Ca}(\text{NO}_3)_2$  and  $\text{MgSO}_4$ , solution 17 had a molecular proportion of 4:2:2, and solution 20 one of 5:2:1. Since solutions 19 and 20 were outstanding in the fruiting phase from the viewpoint of yield of grain, a solution such as 20 might be considered from these results the best solution for all stages of growth. All of these solutions named gave higher yields than the control solution of Shive's having the molecular proportion 3.77:1.09:3.14.

Considering the fruiting phase, since solutions 3 and 10 are so outstanding in producing a yield of grain as well as solution 19 or 20, and not markedly inferior to other solutions having high proportions of phosphorus and potassium, the fact is apparent that at maturity, a high proportion of nitrogen, compared to the phosphorus and potassium carriers, gives as high a yield of grain as a low molecular proportion of nitrogen and high proportion of phosphorus and potassium.

### SUMMARY

These results emphasize the importance of nitrogen in the development of the wheat plant, and indicate that when there is a sufficient supply of nitrogen in the soil the plant will produce a maximum yield with less of the elements containing phosphorus and potassium. In practice, this means that where these elements are known to be deficient, smaller applications of fertilizers containing them are required if there is a liberal supply of available nitrogen. The nitrogen can of course be maintained or supplied by the use of legumes in the rotation, by green manure crops, and by the utilization of farm manures and crop residues.



# STUDIES ON NONARSENICAL STOMACH-POISON INSECTICIDES<sup>1</sup>

By WILLIAM MOORE and F. L. CAMPBELL, *Fruit Insect Investigations, Bureau of  
Entomology, United States Department of Agriculture*

## NATURE OF THE STUDY

In attempts to find a satisfactory insecticide for the Japanese beetle at River-ton, N. J., a rather hasty survey of possible arsenical substitutes was made, and many compounds too expensive for practical use were tested with the idea of helping to develop useful laws of insect toxicology, a subject exceedingly obscure at present.

## PROCEDURE

Since the urgency of the situation would permit no loss of time over unpromising compounds, the method of experimentation chosen does no more than indicate those compounds worthy of more exhaustive tests. All experiments were carried out in small cages; in summer, in the open air under a canvas shelter; in spring and fall, in a greenhouse. A certain number of insects, usually 20, were placed in a cage, and the sprayed or dusted foliage of either potted plants or freshly cut shoots, placed in water, was then introduced. In every case sprayed foliage was allowed to dry before it was placed in a cage. A record was kept of the compound used; its concentration, if used as a spray, in terms of pounds of the insecticide per 50 gallons of water; the number of insects in the cage; the number dead at the end of every 24-hour period after the start of the experiment until it seemed unnecessary to continue it; the percentage killed during seven days, or less, if the test was not run so long; the amount of feeding; and injury to the foliage by the insecticide. In some cases the number of insects that were on the floor of the cage stupefied was also recorded. The extent of feeding was recorded as "slight" if the area eaten was hardly noticeable, "heavy" if the feeding was about the same as that on the control plant, and "medium" if the amount consumed was between the two extremes. In a like manner foliage injury was recorded as "slight" if it could be detected only on close examination, "severe" if all the leaves were practically destroyed, and "medium" if the injury was partial. When the Japanese beetle was not obtainable, tests were made on the tent caterpillar (*Malacosoma americana* Fabricius), the Colorado potato beetle (*Leptinotarsa decemlineata* Say), and the squash lady-beetle (*Epilachna borealis* Fabricius).

## DISCUSSION OF EXPERIMENTS AND TABLES

### INORGANIC COMPOUNDS

Table I shows the effect of certain relatively insoluble salts on the tent caterpillar. The more soluble compounds were the more injurious to both foliage and caterpillar. Barium carbonate and barium oxalate were tested later on the Japanese beetle (Table II), but were without effect. As a general rule the beetle was more resistant than the other insects to the compounds tested. Antimony, though similar chemically to arsenic, was ineffective in both compounds tested.

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<sup>1</sup> Received for publication March 17, 1924.

TABLE I.—Results, against the tent caterpillar, of spraying wild-cherry foliage with inorganic nonarsenical compounds

Compound	Rate of application (pounds per 50 gal- lons water)	Number of insects		First day		Sec- ond day		Third day		Fourth day		Fifth day		Sixth day		Sev- enth day		Per cent killed	Feeding	Foliage injury
		Dropped	Dead	Dropped	Dead	Dropped	Dead	Dropped	Dead	Dropped	Dead	Dropped	Dead	Dropped	Dead	Dropped	Dead			
Barium oxalate...	5	74	54	0	53	0	41	11	30	33	22	8	---	---	17	5	77	Medium	Slight.	
Barium carbon- ate.	5	46	39	0	32	0	26	4	17	7	11	16	---	---	9	3	65	do	Do.	
Barium fluorid...	5	45	12	0	10	0	13	14	4	11	3	0	---	---	5	3	62	Slight	Do.	
Barium sulphate.	5	56	1	0	1	0	0	0	---	---	---	---	---	---	---	---	0	Heavy	None.	
Barium chrom- ate.	5	46	2	0	2	0	3	0	---	---	---	---	---	---	---	---	0	do	Do.	
Barium stearate...	5	65	0	0	0	0	8	0	2	0	0	0	0	0	0	0	0	do	Do.	
Calcium fluorid...	5	139	9	0	3	0	0	0	1	1	0	0	0	0	4	11	9	Slight	Severe.	
Calcium oxalate...	5	37	0	0	0	0	0	0	0	0	0	0	0	0	---	---	0	Heavy	None.	
Lead fluorid.....	5	46	0	0	1	0	1	0	0	0	0	6	---	---	1	11	37	Slight	Slight.	
Lead chromate...	5	78	0	0	0	0	0	4	0	0	0	4	---	---	0	1	12	Medium	None.	
Zinc fluorid.....	5	116	4	0	2	0	7	13	9	14	0	22	---	---	0	42	78	Slight	Medium.	
Control.....	---	97	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Heavy	---	

TABLE II.—Results, against the Japanese beetle, of spraying or dusting smartweed with inorganic nonarsenical compounds

Compound	Rate of application (pounds per 50 gal- lons water)	Number of insects	Number dead							Per cent killed	Feeding	Foliage injury
			First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day			
Barium oxalate.....	5	20	0	0	0	2	0	2	0	20	Heavy...	None.
Barium carbonate.....	5	20	0	0	0	0	0	0	---	0	do.....	Do.
Bismuth carbonate.....	2	20	0	0	1	0	0	---	---	5	do.....	Do.
Lead antimonate.....	2	20	1	0	0	---	---	---	---	5	do.....	Do.
Do.....	5	20	0	0	1	0	---	---	---	5	do.....	Do.
Antimony trisulphid.....	2	20	0	0	1	---	---	---	---	0	do.....	Do.
Do.....	5	20	0	0	---	---	---	---	---	0	do.....	Slight.
Sodium fluorid.....	(a)	20	3	11	---	---	---	---	---	70	Slight...	Severe.
Control.....	---	20	0	0	0	0	0	0	0	0	Heavy...	---

a Dusted.

ORGANIC COMPOUNDS

CYANIDS

Considerable work was done on simple and complex metallic cyanids with the dea of finding a cyanid that would be comparatively stable on foliage, and yet would break down in the alimentary canal of the insect with the liberation of deadlly hydrocyanic acid.

The so-called lead cyanid is not a simple cyanid of the formula Pb(CN)<sub>2</sub>, but is a basic salt containing but a small percentage of CN, which varies with the concentrations of the solutions used in its precipitation, and with the extent of its exposure to air and moisture after preparation. Statements in several textbooks in regard to this cyanid conflict. The observations of Williams<sup>2</sup> were confirmed by preparation and analysis of the cyanid in the laboratory. The ineffectiveness of this compound was thought to be due to the low percentage of CN and its rapid volatility in the form of HCN.

<sup>2</sup> WILLIAMS, H. E.—THE CHEMISTRY OF CYANOGEN COMPOUNDS. 423 p. London. 1915.

Zinc cyanid has hitherto been considered a stable cyanid, but under spraying conditions it also loses HCN as the spray dries. This was proved by placing a freshly sprayed plant under a large bell jar closed at the top by a two-holed rubber stopper. Air was aspirated from the jar through two Drechsel wash bottles containing a solution of sodium carbonate to absorb HCN. After air had been drawn through the train for several hours, the contents of the wash bottles were analyzed, and HCN was found in the first bottle, but not in the second. This loss of HCN with large surface exposure of the cyanid to air and moisture was further shown by spraying a mixture of the cyanid and water on a glass plate. After the spray deposit had dried, it was scraped from the glass, weighed, analyzed, and compared with the fresh material. In every case there was a considerable loss of HCN. Finally it was proved that under certain conditions a spray deposit of zinc cyanid would lose all its CN. A plate was sprayed with a mixture of the cyanid and water and the spray deposit was allowed to dry. It was then tested qualitatively for CN. The next day it was sprayed with water to simulate dew, and tested again after it dried. The spraying, drying, and testing were repeated daily till no test was obtained for CN; sufficient evidence for the complete hydrolysis of the compound. Examination of Table III shows that, while zinc cyanid was fairly effective against half-grown caterpillars, it did not kill full-grown caterpillars. It was toxic to the potato beetle (Table IV), but hardly even retarded the feeding of the Japanese beetle (Table V).

TABLE III.—Results, against the tent caterpillar, of spraying or dusting wild-cherry foliage with cyanids

Compound	Application		Caterpillars		Number dead							Per cent killed	Feeding	Foliage injury
	Method	Rate (pounds per 50 gallons water)	Number	Stage of growth	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day			
Nickel cyanid...	Dust...	---	40	Half-grown	0	3	3	6	---	---	---	30	Slight...	None.
Cuprous cyanid...	do...	---	40	do	3	13	13	11	---	---	---	100	do	Do.
Zinc cyanid...	do...	---	40	do	0	0	0	16	---	---	---	40	do	Do.
Copper nitroprussid.	Spray...	10	20	do	0	2	1	1	2	0	5	55	do	Do.
Zinc nitroprussid.	do...	10	20	do	0	0	0	0	0	0	0	0	do	Do.
Cuprous cyanid...	Dust...	---	20	Full grown	0	0	2	1	1	7	---	55	do	Do.
Zinc cyanid...	do...	---	20	do	0	0	0	1	1	0	---	10	Medium	Do.
Nickel cyanid...	do...	---	20	do	0	0	0	0	0	0	---	0	Heavy	Do.
Cuprous thio-cyanate.	do...	---	20	do	0	0	3	1	1	6	---	55	Slight...	Do.
Cuprous cyanid...	Spray...	5	20	do	0	0	4	3	1	0	1	45	do	Do.
Zinc cyanid...	do...	5	20	do	0	0	0	0	0	0	0	0	Heavy	Do.
Nickel cyanid...	do...	5	20	do	0	0	0	0	0	0	1	5	do	Do.
Cuprous thio-cyanate.	do...	5	20	do	0	0	10	6	3	---	---	95	Medium	Do.
Lead arsenate...	do...	5	20	do	0	0	2	7	3	4	---	80	Slight...	Do.
Control	do...	---	20	do	0	0	0	0	0	0	---	0	Heavy	---

TABLE IV.—Results, against the Colorado potato beetle, of spraying potato and tomato plants with cyanids

Compound	Application		Number of insects	Number dead							Per cent killed	Feeding	Foliage injury
	Method	Rate (pounds per 50 gallons water)		First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day			
Zinc cyanid	Dust	-----	20	3	0	0	1	0	2	-----	30	Slight	Yellowing.
Cuprous cyanid	do	-----	20	7	2	2	3	0	-----	-----	70	do	None.
Nickel cyanid	do	-----	20	1	0	1	-----	-----	-----	-----	10	None	Wilted.
Copper nitroprussid	Spray	10	20	1	2	0	0	1	-----	0	20	Slight	None.
Zinc nitroprussid	do	10	20	0	3	0	1	1	-----	1	30	do	Do.
Copper nitroprussid	do	5	20	0	0	0	0	0	-----	-----	0	Medium	Do.
Zinc nitroprussid	do	2	20	0	0	0	0	0	-----	-----	0	do	Do.
Ammonium nickel cyanid-aniline.	do	10	20	2	1	0	0	1	-----	2	30	None	Do.
Ammonium nickel cyanid-phenol.	do	10	20	0	0	0	2	0	0	-----	10	Slight	Do.
Ammonium nickel cyanid-benzene.	do	10	20	0	0	0	0	0	0	2	10	do	Do.
Control	-----	-----	20	0	0	0	0	0	0	0	0	Heavy	-----

Nickel cyanid, although stable, as shown by long-continued spraying tests similar to the foregoing on glass plates, was not as toxic as zinc cyanid, and, moreover, did not have the physical properties of a good insecticide.

TABLE V.—Results, against the Japanese beetle, of dusting plants with cyanids

Compound	Number of insects	Number dead				Per cent killed	Feeding	Foliage injury
		First day	Second day	Third day	Fourth day			
Zinc cyanid	20	0	0	0	-----	0	Medium	None.
Cuprous cyanid	20	0	3	1	-----	20	None	Do.
Cuprous oxid	20	0	0	-----	-----	0	Heavy	Do.
Cuprous iodid	20	0	0	0	-----	0	Medium	Do.
Cuprous thiocyanate.	20	0	-----	-----	-----	0	Heavy	Do.
Lead thiocyanate	20	0	-----	-----	-----	0	do	Do.
Lead arsenate	20	1	1	2	-----	20	Slight	Do.
Control	20	0	0	0	-----	0	Heavy	Do.

Cuprous cyanid, CuCN, was tested in the same manner as zinc cyanid and was found to be stable for at least a month, when the glass-plate experiment was discontinued. This cyanid was the only nonarsenical tested which was approximately equal to lead arsenate in toxicity against the Japanese beetle (Table VI). Both the caterpillars and the beetles ate it very sparingly and succumbed quickly. It is hard to wet for a spray mixture. It adheres to foliage excellently as a dust, but is washed off easily by rain. It did not injure the foliage of wild cherry or that of smartweed, but in a special test to determine the point it did injure peach foliage. The writers did not have the opportunity to determine whether or not the burning was due to a trace of the free copper ion.

Since cuprous cyanid was toxic to the Japanese beetle, and nickel cyanid equally stable, was not, it seemed possible that Cu was responsible for the toxicity rather than CN. Therefore other cuprous compounds were prepared and compared with cuprous cyanid. On tent caterpillars cuprous thiocyanate showed remarkable toxicity. It was even more toxic than lead arsenate applied at the same rate. But hopes for its success against the beetle were vain. It

failed completely, as did the other cuprous compounds and another thiocyanate. All efforts to attribute the toxicity of cuprous cyanid to one ion or the other proved fruitless. It is but another of the many instances which show how far we are from understanding the relation between toxicity and the chemical constitution of insecticides.

TABLE VI.—Results, against the Japanese beetle, of spraying smartweed with cuprous cyanid and lead arsenate

Compound	Rate of application (pounds per 50 gallons water)	Number of insects	Number dead							Per cent killed	Feeding	Foliage injury
			First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day			
Cuprous cyanid.....	2	20	0	0	1	3	3	7	---	70	Medium..	None.
Do.....	3	20	1	2	1	0	0	9	---	65	Slight....	Do.
Do.....	4	20	0	0	1	1	1	6	---	45	do.....	Do.
Do.....	5	20	0	2	0	1	2	6	---	55	do.....	Do.
Lead arsenate.....	2	20	0	2	0	1	4	3	---	50	Medium..	Do.
Do.....	3	20	0	2	3	0	4	6	---	75	do.....	Do.
Do.....	4	20	0	0	3	2	5	4	---	70	Slight....	Do.
Do.....	5	20	1	2	2	4	2	2	---	65	do.....	Do.
Control.....	2	20	0	0	0	0	0	0	---	0	Heavy....	

The nitroprussids of copper and zinc were prepared and tested. Their toxicity was so low that no effort was made to work out the disposal of CN.

Hofmann and Höchtlen<sup>3</sup> describe additive compounds formed by the addition of benzene, phenol, or aniline to an ammoniacal solution of nickel cyanid. The compounds break down into their component parts on the addition of dilute acid or alkali. With a "shot-gun" remedy of such properties it seemed almost impossible to miss the beetle, but the compounds showed only low toxicity.

#### ABSORBED ORGANIC COMPOUNDS

It was thought that gaseous, liquid, or soluble solid organic compounds which might be destructive to plant tissues could be applied if they were absorbed on inert materials like clay; it being assumed, of course, that the insect would liberate the adsorbed toxic compound. Unfortunately there was not time to test the assumption by using an adsorbed compound of known toxic properties and of known concentration on the inert material. A series of clays and charcoals was prepared on which such substances as carbazol, anthracene, quinine, amyl alcohol, and dimethylaniline had presumably been adsorbed. They were entirely without effect; but since the idea was not given a fair trial, it is still worth considering.

A silica gel and a charcoal, on which three war gases and arsenious oxid had been adsorbed, were obtained from the United States War Department. The percentage of each poison in the preparations was given. The gases were not held very tightly at the temperature at which the dusting was done, because their odor was apparent at a distance from the unstoppered bottles. Nevertheless, none of the war gases had the slightest effect on insects as susceptible as the potato beetle and the tent caterpillar (Table VII). Moreover, phosgene, or perhaps its decomposition product HCl, burned the foliage severely. Arsenious oxid burned the foliage as severely as if it had been in water solution.

<sup>3</sup> HOFMANN, K. A., and HÖCHTLEN, F. ABNORME VERBINDUNGEN DES NICKELS. Ber. Deut. Chem. Gesell. 36: 1149-1151. 1903.



TABLE VII.—Results, against the tent caterpillar and the Colorado potato beetle, of dusting wild-cherry foliage and tomato plants with organic compounds on which war gases had been adsorbed

## TENT CATERPILLAR ON WILD-CHERRY FOLIAGE

Dusts	War gas	Per cent	Number of insects	Number dead					Per cent killed	Feeding	Foliage injury
				First day	Second day	Third day	Fourth day	Fifth day			
Silica gel.....	Phosgene.....	3.0	20	0	0	0	0	0	0	Heavy.....	None.
Do.....	Chloropicrin.....	20.0	20	0	0	0	0	0	0	do.....	Severe.
Do.....	Chloracetophenone.....	12.5	20	0	0	0	0	0	0	do.....	Slight.
Do.....	Arsenious oxid.....	5.0	20	0	12	0	3	0	75	Slight.....	Severe.
Charcoal.....	Phosgene.....	25.0	20	0	0	0	0	0	0	Heavy.....	Do.
Do.....	Chloropicrin.....	35.0	20	0	0	0	0	0	0	do.....	None.
Do.....	Chloracetophenone.....	11.0	20	0	0	0	0	0	0	do.....	Do.
Do.....	Arsenious oxid.....	9.0	20	0	11	2	4	0	85	Slight.....	Severe.
Control.....			20	0	0	0	0	0	0	Heavy.....	

## COLORADO POTATO BEETLE ON TOMATO PLANTS

		<i>Per cent</i>										
Silica gel	Phosgene	3.0	20	0	0	0	0	0	0	Heavy	None.	
Do	Chloropicrin	20.0	20	0	0	0	0	0	0	Medium	Do.	
Do	Chloracetophenone	12.5	20	0	0	0	0	0	0	Heavy	Do.	
Do	Arsenious oxid	5.0	20	0					0	None	Severe.	
Charcoal	Phosgene	25.0	20	0					0	do	Do.	
Do	Chloropicrin	35.0	20	0	0	0	0	0	0	Heavy	None.	
Do	Chloracetophenone	11.0	20	0	0	0	0	0	0	do	Do.	
Do	Arsenious oxid	9.0	20	0					0	None	Severe.	
Control			20	0	0	0	0	0	0	Heavy	None.	

## ALKALOIDS

The alkaloids listed in Table VIII were ineffective. The unexpected resistance of insects to these alkaloids has also been observed by Crozier <sup>4</sup> and by Richardson and Smith.<sup>5</sup> The reason for the experiments with alkaloids was detailed in another paper by Moore.<sup>6</sup>

TABLE VIII.—Results, against the Japanese beetle, of spraying smartweed plants with alkaloids

Compound	Rate of application (pounds per 50 gallons water)	Number of insects	Number dead						Per cent killed	Feeding	Foliage injury
			First day	Second day	Third day	Fourth day	Fifth day	Sixth day			
Atropine sulphate.....	0.001	20	0	0	0	0	0	0	0	Heavy.....	None.
Strychnine sulphate.....	.002	20	0	0	0	0	0	0	0	do.....	Do.
Morphine sulphate.....	.005	20	0	0	0	0	0	0	0	do.....	Do.
Do.....	.005	20	2	2	0	0	0	0	20	do.....	Do.
Veratrin.....	.005	20	0	0	0	1	0	0	5	do.....	Do.
Control.....		20	0	0	0	0	0	0	0	do.....	Do.

<sup>4</sup> CROZIER, W. J. REVERSAL OF INHIBITION BY ATROPINE, IN CATERPILLARS. Biol. Bul. 43:239-245, 1922.

<sup>5</sup> RICHARDSON, C. H., and SMITH, C. R. STUDIES ON CONTACT INSECTICIDES. U. S. Dept. Agr. Bul. 1169, 15 p. 1923.

<sup>6</sup> MOORE, W. THE REACTION OF THE JAPANESE BEETLE TO ARSENICAL SPRAY DEPOSITS. Jour. Econ. Ent. 15: 67-70. 1922.

INSOLUBLE AROMATIC COMPOUNDS

Certain organic compounds insoluble in water can be dusted on foliage without injury to it. Carbazol, acetylsalicylic acid, and acetanilid when so used were quite effective against the Japanese beetle (Table IX), but the season was nearing its close and the lowered resistance of the beetles may have accounted for it. Further tests, however, seemed worth while and were carried out later in the greenhouse on the squash lady beetle (Table X). The effect of various organic compounds on bean foliage is recorded in Table XI.

TABLE IX.—Results, against the Japanese beetle, of dusting smartweed plants with insoluble aromatic compounds

Compound	Number of insects	Number dead				Per cent killed	Feeding	Foliage injury
		First day	Second day	Third day	Fourth day			
Carbazol.....	20	0	1	7	-----	40	Heavy.....	None.
Acetanilid.....	20	0	6	11	-----	85	Slight.....	Slight.
Acetylsalicylic acid.....	20	0	12	8	-----	100	do.....	Do.
Control.....	20	0	0	2	-----	10	Heavy.....	

TABLE X.—Results, against the squash lady beetle, of dusting pumpkin plants with insoluble aromatic compounds

Compound	Number of insects	First day		Second day		Third day		Fourth day		Fifth day		Sixth day		Seventh day		Per cent killed	Remarks
		Dropped	Dead	Dropped	Dead	Dropped	Dead	Dropped	Dead	Dropped	Dead	Dropped	Dead	Dropped	Dead		
Anthracene.....	20	---	0	---	0	---	0	---	0	---	0	---	0	---	---	0	Beetles were stupefied temporarily.
Anthraquinone.....	20	---	0	---	0	---	0	---	0	---	0	---	0	---	---	0	Plants readily eaten without noticeable effect on beetles.
Tolidin.....	20	---	0	---	0	---	0	---	0	---	0	---	0	---	---	0	Do.
Phenyl- $\alpha$ -naphthylamin. <sup>a</sup>	20	---	0	---	0	---	0	---	0	---	0	---	0	---	---	0	Beetles were stupefied temporarily.
Acetyl-ortho-amino phenol.	20	---	0	---	0	3	0	2	0	---	2	---	---	---	---	10	Slow action; no striking effect.
2-6 dichloro-4-nitranilin.	20	---	1	4	0	---	0	---	0	---	0	---	0	0	0	5	No noticeable effect.
Diacetyl-ortho-phenylenediamin.	20	---	0	---	0	---	0	---	0	2	0	4	2	---	8	50	No striking effect.
4 nitro 2-aminotoluene	20	7	0	16	0	17	0	17	0	18	0	12	0	13	0	0	Beetles were stupefied; 13 finally died.
Ferric - para - nitrobenzoate.	20	---	0	---	0	---	0	---	0	---	---	---	---	---	---	0	Plants readily eaten without noticeable effect on beetles.
3-nitro-4-amino-toluene.	20	3	0	1	0	4	0	6	0	7	0	7	0	---	---	0	Somewhat similar to 4-nitro 2-aminotoluene.
Control.....	20	---	0	---	0	---	0	---	0	---	0	---	0	---	---	0	

<sup>a</sup> Diluted one-half with clay.

TABLE XI.—*Effect of various organic compounds on bean foliage*COMPOUNDS HAVING THE NH<sub>2</sub> GROUP

Compound	Effect on foliage	Compound	Effect on foliage
Meta-nitroanilin.....	Medium burning.	Acetyl-para aminophenol.....	No burning.
2-4-Dichloranilin.....	Serious burning.	Acetyl-para methylamino-phenol.....	Do.
2-4-6 Trichloranilin.....	Medium burning.	Anthranilic acid.....	Serious burning.
2-6 Dichlor-4-nitroanilin.....	No burning.	Para-aminobenzoic acid.....	Medium burning.
Para-chloracetanilid.....	Serious burning.	Acetyl-para-aminobenzoic acid.....	No burning.
Para-nitroacetanilid.....	Do.	Benzamid.....	Serious burning.
Para-phenylenediamin.....	Do.	Aminosalicylic acid.....	No burning.
Meta-tolylenediamin.....	Do.	Alpha-naphthylamin.....	Serious burning.
Para-aminoacetanilid.....	Do.	Beta-naphthylamin.....	Medium burning.
Diacetyl-ortho-phenylenediamin.....	No burning.	Phenyl-alpha-naphthylamin.....	Serious burning.
2-Diaminochlorbenzene.....	Serious burning.	Nitronaphthylamin.....	Do.
Para-toluidin.....	Do.	Diphenylamin.....	Do.
Acetyl-ortho-toluidin.....	Do.	Acetyldiphenylamin.....	Do.
Acetyl-para toluidin.....	Do.	Carbazol.....	No burning.
3-Nitro 4-aminotoluene.....	Do.	Benzidin.....	Medium burning.
4-Nitro 2 aminotoluene.....	No burning.	Tolidin.....	No burning.
Ortho-aminophenol.....	Serious burning.	Benzanilid.....	Do.
Para-aminophenol.....	Medium burning.	Oxanilid.....	Do.

COMPOUNDS CONTAINING THE COOH GROUP BUT NOT THE NH<sub>2</sub> GROUP

Para-chlorbenzoic acid.....	Serious burning.	2-4-6 Trinitrobenzoic acid.....	Serious burning.
Para-nitrobenzoic acid.....	Do.	5-Nitrosalicylic acid.....	Do.
2-4-Dinitrobenzoic acid.....	Do.	Cresotinic acid.....	Do.
3-5-Dinitrobenzoic acid.....	Medium burning.	Para-cyanobenzoic acid.....	No burning.

COMPOUNDS CONTAINING THE OH GROUP BUT NOT THE NH<sub>2</sub> OR COOH GROUP

Para-nitrophenol.....	Serious burning.	Alpha-naphthol.....	Serious burning.
Para-nitrophenetol.....	Do.	Beta-naphthol.....	Do.
3-5 Nitro-ortho-cresol.....	Do.		
Trinitro-meta-cresol.....	Medium burning.		

COMPOUNDS CONTAINING THE NO<sub>2</sub> GROUP BUT NOT THE NH<sub>2</sub>, COOH, OR OH GROUPS

Para-nitrochlorbenzene.....	Serious burning.	Dinitrotoluene.....	Serious burning.
Para-chlor-nitrobenzene.....	Do.	Alpha-nitronaphthalene.....	Do.

## COMPOUNDS CONTAINING THE CO GROUP

Anthraquinone.....	No burning.	Phthalic anhydrid.....	Serious burning.
Benzil.....	Do.		

## HYDROCARBONS

Diphenyl.....	Serious burning.	Anthracene.....	No burning.
Phenanthrene.....	Do.		

## MISCELLANEOUS COMPOUNDS

Hexachlorethane.....	Serious burning.	Thio-beta-naphthol.....	Serious burning.
Dichlordiethyl sulphorid.....	Do.	Triphenyl arsine.....	Medium burning.

## SUMMARY

No inorganic nonarsenical was found of noteworthy toxicity to the Japanese beetle.

The relative stability of simple metallic cyanids was determined in efforts to explain the differences in toxicity among them. When spread out, as in a spray deposit, copper and nickel cyanids are stable, zinc cyanid breaks down slowly, and so-called lead cyanid hydrolyzes quickly.

Copper cyanid was the only nonarsenical tested the toxicity of which was comparable to that of lead arsenate on the Japanese beetle.

Copper thiocyanate showed high toxicity to tent caterpillars, but was non-toxic to the Japanese beetle.

Complex cyanids and other rather complex insoluble organic compounds exhibited low toxicity, and were interesting only from a theoretical point of view.

No definite relations were found between chemical constitution and toxicity.

# JOURNAL OF AGRICULTURAL RESEARCH

## CONTENTS

	Page
Studies upon the Relative Merits of Sweet Corn Varieties for Canning Purposes and the Relation of Maturity of Corn to the Quality of the Canned Product C. W. CULPEPPER and C. A. MAGOON	403
The Photoperiodism of <i>Tephrosia candida</i> T. B. McCLELLAND	445
Antineuritic Vitamin in Poultry Flesh and Eggs RALPH HOAGLAND and ALFRED R. LEE	461
Dissemination of the Stem and Bulb Infesting Nematode, <i>Tylenchus dipsaci</i> , in the Seeds of Certain Composites G. H. GODFREY	473
Some Modifications of the Picric-Acid Method for Sugars J. J. WILLAMAN and F. R. DAVISON	479
Effect of Moisture on a Seed-Borne Bean Disease LEWIS T. LEONARD	489

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## STUDIES UPON THE RELATIVE MERITS OF SWEET CORN VARIETIES FOR CANNING PURPOSES AND THE RELATION OF MATURITY OF CORN TO THE QUALITY OF THE CANNED PRODUCT<sup>1</sup>

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### INTRODUCTION

The market value of a canned food is determined primarily by its quality. Any factors, therefore, which affect the quality of a product deserve careful study to the end that a high standard of excellence may be established and consistently maintained. In the canning of sweet corn the variety factor, together with climatic considerations, has been regarded as of great importance in determining the quality of the finished product, and certain varieties have been thought by some to be exceptionally desirable for canning. This fact has had no small influence in directing the progress of corn canning both as a home and a commercial enterprise. Many kinds of sweet corn are grown in this country and a considerable number of them are being canned. It has seemed desirable, therefore, to subject the more widely known of these to careful comparative study to learn the importance of variety as a factor in canning quality.

Since certain factors other than varietal differences in the corn are known to affect the quality of the canned product, as for instance, the stage of ripeness, the promptness in handling after plucking from the stalk, etc., it was thought wise to give particular attention also to some of these matters and to record such related data as the conditions of the experiment permitted. This report, therefore, will be found to deal with matters of interest not only to growers and canners of corn but also to those who are interested in the physical development of the corn and in the chemical transformations taking place during the growing and maturing of the ear.

### REVIEW OF LITERATURE

Sweet corn was first grown specifically for canning purposes about 1842, when Isaac Winslow, the pioneer in corn canning, arranged with his brother-in-law Caleb Jones, "to plant a piece of green corn for experimental purposes" (18).<sup>2</sup> It was 10 years later, however, before corn canning was well established, though an invoice of sale of canned corn as early as 1848 is on record.

The first comprehensive study of sweet corn seems to have been made by Salisbury (54) in 1849. This writer made observations upon 20 varieties of corn, including sweet, pop, dent, and flint varieties. He kept careful notes upon their characteristics as shown in the field and recorded the results of numerous chemical analyses made during the growth of the corn. In 1885, apparently receiving more or less inspiration from the work of Salisbury mentioned above, Körnicke and Werner (35) in their volumes upon the cereal grains discussed in considerable detail the subject of corn varieties.

<sup>1</sup> Received for publication Mar. 11, 1924.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 440-443.

The establishment of the State agricultural experiment stations in 1887 seems to have greatly stimulated studies upon corn, and during the next 10 or 12 years numerous papers were issued from different States dealing with various phases of the subject. These had to do with field observations upon the physical characteristics of the plants, earliness and lateness in maturing, yields, etc., with the taxonomy and description of varieties, the character and proportions of the various chemical constituents of the grain, the transformations in chemical composition during growth, breeding experiments, etc. It is manifestly impossible at this time to review all this work in detail and references to the literature will be confined, for the most part, to those papers dealing primarily with sweet corn.

Among those who have made more or less careful field studies upon sweet corn varieties may be mentioned Burrill and McCluer (12, 13), Butz and Baldwin (16), Latta (36), Taft, Coryell, and Gladden (67), Munson and Gould (42), Butz (15), Taft, Gladden, and Hedrick (68), Gould (26, 27), Rane (48) and Price and Drinkard (47). A brief taxonomic grouping of sweet corn varieties based upon careful field observations was made in 1890 by Burrill and McCluer (13) and in 1899 Sturtevant (66) prepared a monograph dealing with some 800 varieties in which an attempt was made to place the nomenclature upon a sound scientific basis. This monograph is regarded as the best treatment of the subject ever undertaken and is recognized as a standard work.

The literature bearing upon the breeding of sweet corn for the last 30 years has been confined largely to the genetics of the subject. Little has been written concerning the improvement of strains and the production of new varieties. However, this does not mean that such work has not been done but rather that the investigators have failed to record their results. Advance in this work has been made along three lines, namely, the development of superior varieties by hybridization, improvement by careful selection of existing strains, and the development of first-year hybrids from pure self-fertilized strains. The first has resulted in new types with respect to color, arrangement and size of grains, improved yields, etc.; the second, by use of the ear-to-row or "remanent" method, has yielded superior strains with respect to size and number of ears, etc.; and the third promises much in the way of uniformity of ears and yields.

For many years the practical breeding and selection of improved varieties of sweet corn have been largely in the hands of commercial seedsmen, though several of the State agricultural experiment stations are continuing work along this line.<sup>3</sup> Among those who by their early studies contributed materially to the success of later workers in this field may be mentioned Beal (6, 7, 8, 9), Ingersoll (32), Sanborn (55), McCluer (38), Morrow and Gardner (40, 41), Williams (72), Shull (58, 59) and East (21). The late Dr. W. W. Tracy, sr., of this office, whose breeding studies were never put into printed form, was likewise a potent factor in the development of improved strains. Reference to papers upon more recent investigations will be made in the discussion of results of the present study.

The chemistry of corn is a subject that has engaged the attention of many workers. Gorham (25), as far back as 1821, during his studies upon the composition of corn, discovered zein as one of the chemical constituents of the kernel, and Bizio (10) discovered the presence of oil. Boussingault (11) made determinations of the nitrogen content and pointed out the possibility of variations in the chemical constituents of the grain as being due to climatic factors. Horsford (31) reported complete ultimate analyses. Salisbury (54) whose comprehensive study has already been cited, was followed by a number of European workers,

<sup>3</sup> Work upon sweet corn improvement is being done at stations in Georgia, Wisconsin, Maine, Ohio, Connecticut, Iowa, Maryland, Indiana, Louisiana, New York, and other States.

including Polson (46), Poggiale (45), Stepf (61), Fresenius (24), and by Jackson (33) in this country, who studied the chemistry of the corn kernel. With the exception of Salisbury, however, the work of these early investigators was concerned with field varieties only. Atwater (5) in 1869 reported the results of analyses upon several varieties of corn, including one of sweet corn.

Hornberger (30) in 1882 added materially to the literature upon the chemistry of corn by publishing a very comprehensive paper, giving special attention to the chemical history of the plant during growth. While this work was done upon a single variety of field corn (Badische Frühmais, Poppelsdorf 1878) it opened up a very important field of research which has not yet been completely covered. In 1883 Richardson (50) published the results of investigations upon wheat and corn in which are compiled the analyses of 19 samples of sweet corn derived from various sources, and again in 1884 (51) figures upon 4 others were placed on record. Flechsig (23) in 1886 made analyses of 14 different varieties of corn, including one sweet variety. Schweitzer (56), following the same line of investigations as Hornberger, in 1889 reported upon a study of the life history of corn at its different periods of growth, using the St. Charles White as the source of his material. In the same year Failyer and Willard (22) reported upon analyses of King Philip and Yellow Dent corns at different stages of growth, and Washburn (70) published his results upon the changes in chemical composition of sweet corn during the ripening processes.

Further work upon the chemistry of the corn kernel was reported by Maynard (39), but his analyses were based upon air-dried material, hence the figures are difficult of comparison. Chittenden and Osborne (17) gave special attention to the proteins of the kernel, and later on Osborne and Clapp (44) carried the investigation of the proteins still further, making special study of the amino acids resulting from hydrolysis. The characteristic proteins in high and low protein corn have been more recently considered by Showalter and Carr (57). The Report of the Connecticut State Agricultural Experiment Station for 1893 (19) recorded the results of analyses upon 90 samples of corn kernels and pointed out the relation of climatic and soil factors to the chemical composition of the grain. Stone (63) in his study of the carbohydrates from various materials considered somewhat those of maize, but feed varieties only seem to have been examined. Hopkins (29) went rather carefully into the chemistry of the corn kernel and in addition to a critical review of the literature recorded the results of his own studies upon the constituents of corn, giving special attention to the oil. The major portion of this work, at least, seems to have been done upon a single variety of field corn (Burr's White) and apparently nothing was done upon sweet varieties.

Of very great interest and importance to the present work is that which was reported by Straughn (64) in 1907. The object of this work as stated by the writer was to assist in the breeding of superior sweet corn for table and canning uses. He found that so constant and uniform was the sugar of the mature grains that it was not feasible to select for high sugar content from the dry kernels. The wrinkling of the kernel seemed to bear some relationship to the sugar content, however, the finely wrinkled grains having a slightly higher sugar content than the more coarsely wrinkled. Of particular importance in this work was the finding that the sugar content of the corn in edible condition alters rapidly during the first few hours after the ears are plucked from the stalk. Further studies upon the transformations occurring in green sweet corn during storage at different temperatures were reported by Straughn and Church (65) in 1909. Losses in sugar and deterioration in flavor were noted at all temperatures and their conclusions were summarized in the statement that corn should be put on the market or canned with as little delay as possible after gathering.



This work, so well begun at the Maryland State Experiment Station, has been continued and during the last few years several papers have appeared which have gone further into the subject and added materially to the solution of the corn canning problem. Thus, Appleman and Arthur (2) have reported upon carbohydrate metabolism in green sweet corn during storage at different temperatures. These workers followed the rate of loss of sugars and found that the loss was doubled for every increase of 10° up to 30° C., until it reached 50 per cent of the initial total sugar and 60 per cent of the sucrose. The rate of loss was slower beyond that point. They found this loss in sugar to be effected in small part by respiration but primarily by its condensation into polysaccharides, chiefly starch. The importance of respiration which causes the inside heating of large piles of corn as being responsible for losses of sugar was emphasized. Similar results were obtained by Stevens and Higgins (62) who conducted studies upon sweet corn grown in both Maryland and Maine. In 1921 Appleman and Eaton (3) published upon the evaluation of climatic temperature efficiency for the ripening processes in sweet corn, recording considerable analytical data showing changes in the composition of corn during the ripening period, and predicting the average expectations as to the time between premilk and best edible milk stage, and the length of time in the best edible stage for different ripening seasons in various Eastern States. This valuable work would have been much more so if the exact age of the ears at the various sampling dates had been known. The same year Appleman (1) recorded experiments designed to determine the reliability of the nail test for predicting the chemical composition of green sweet corn. It was found that the reliability of the test was influenced by the rate of ripening and the rate of water loss by evaporation, the test being most reliable when applied to crops which ripen slowly in the cool autumn.

This brief survey of the literature is not intended as a complete discussion of all the valuable work that has been done upon corn but rather as showing the nature and scope of it. It is impossible in a paper of this size to discuss in detail the work of more than a few writers. It is believed, however, that the work cited fairly represents the nature and quality of what has been done along these lines. More specific reference will be made to these and other papers in the discussion of results of the present investigations. In reviewing these papers it is seen that the taxonomy of sweet corns has received considerable attention, the behavior of the different varieties under field conditions has been widely studied, and the selection and breeding of improved varieties have reached a point of scientific accuracy. The organic and mineral constituents of the kernel have been investigated by numerous workers, and an attempt made to follow through chemically the life history of the corn. The relation of temperature and other factors to the ripening of the corn, and the effects of storage upon table qualities have been the subject of careful research. A comprehensive study of sweet corn varieties from the standpoint of their desirability for canning purposes, however, seems never to have been attempted, and several matters of fundamental importance to corn canning have not yet been fully investigated. No basis seems to have been worked out for determining the relative merits of different varieties, and just what constitutes quality is but vaguely understood. The fundamental differences between field and sweet varieties as affecting canning quality, though perhaps appreciated, have never been submitted to careful investigation. Much still remains to be done upon the history of the chemical transformations undergone during the development of the ear and no more accurate field method for the determination of maturity of the corn has yet been devised than the actual "rule of thumb" method known as the nail test. The relation of meteorological and other climatic or regional factors, while recognized, have not been sufficiently investigated.

These and related considerations have led to the present investigations, and these data are presented in the hope that they may be of assistance in solving some of the problems of corn canning.

## EARLY INVESTIGATIONS

The experimental work upon which this report is based was begun in 1919 when a number of the leading varieties of sweet corn were tested for the comparative merits of their canned product. The corn was grown especially for the work at the Arlington experiment farm, Virginia. When sufficiently mature, the ears were plucked in what was considered prime canning condition, and removed at once to the laboratory where the corn was immediately prepared for the can. Uniform canning methods were followed throughout the whole experiment so that the canned products were entirely comparable. Shortly after the end of the season these canned corns were subjected to critical comparative study with respect to appearance, flavor, and general table qualities, and the results placed on record for future reference in connection with later experiments similarly conducted.

During the season of 1920 the work was repeated, using a somewhat larger number of varieties. Upon critical examination of the cut-out material of this pack, however, it was found that although the corns had been grown under as nearly identical conditions as possible, and the same canning methods had been followed, the corns graded entirely differently from those of the preceding season. For instance, Country Gentleman which graded "first" in the 1920 pack held third place the previous year, and Golden Bantam which was given first place in 1919 yielded to Charlevoix in 1920. Other varieties also held different relative positions.

Reserve stocks of the 1919 pack were then opened for comparison with the corns canned in 1920, and the packs subjected to careful study to determine what the nature of the differences were, and to learn, if possible, the reasons for the same. The conclusion arrived at was that some factor or factors other than varietal differences determine to a large extent the quality of the canned corn. The most prominent factor seemed to be that of maturity of the corn at the time of harvesting which affected the condition of the carbohydrates, the delicacy of flavor, and the toughness of the kernel. It was thought also that climatic or seasonal factors might be responsible in part, and possibly also the soil conditions. It was apparent that here was a very important problem which needed thorough study.

Pressure of work upon other investigations prevented the carrying forward of this work during the 1921 season.

## WORK OF 1922

In the spring of 1922 plans were laid for the study of the problem upon a more comprehensive scale, the object being to correlate varietal, maturity, and other factors involving the quality of the canned product, and to bring to light any significant facts previously neglected or overlooked. The plan called for the study of all the important varieties used for canning purposes, under, as nearly as possible identical conditions as regarded climate, soil and cultivation, and field and laboratory technique. No attempt was made to investigate the relation of fertilizer and soil factors to the problem but meteorological and other environmental factors were studied so far as data in regard to them could be obtained.

The varieties studied included both white and yellow sorts and comprised early, medium and late maturing corns. These, given in approximately the order of maturing, were: Of the white sorts, Howling Mob, Crosby, Hickox's Improved, Potter's Excelsior, Kelly's Hybrid, Mammoth Sugar, Old Colony, Narrow Grained Evergreen, Country Gentleman, and Stowell's Evergreen; and of the yellow, Golden Bantam, Dreer's Golden Giant, Charlevoix, Morse's Golden

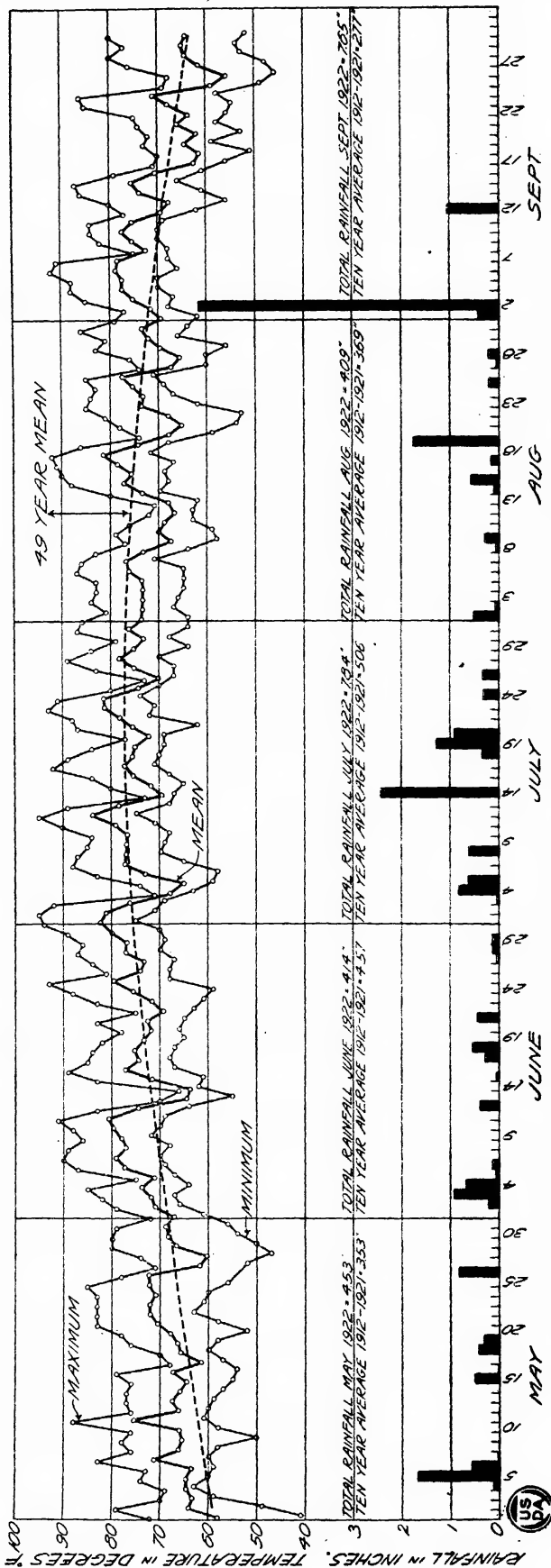


Fig. 1.—Curves and graphs showing temperature and rainfall data covering the period of the tests together with long period averages for the same

Cream and Vaughn's Bantam Evergreen. Reid's Yellow Dent and Boone County White, two standard varieties of field corn, were grown along with the sweet varieties and these were studied for comparison with the sugar corns throughout the entire experiment.

Golden Bantam and Charlevoix of the yellow varieties and Howling Mob and Crosby of the white varieties were planted May 17, 1922. All the others were planted May 24, 1922. The soil was very uniform, deep, rich loam and the plots themselves level and well drained by an underground tile system. The kind and amount of fertilizer and the nature and amount of cultivation were the same for all plots. With the exception of Country Gentleman, which was more heavily seeded and later required thinning, the seeding was uniform for all and a good, uniform stand was obtained in practically all cases. The seed was purchased from commercial concerns which regularly supply the sweet corn growers and it is believed fairly represented that generally sold for canning purposes.

### METHODS

In order that the samples of corn from the different plots might be strictly comparable it was essential that the material from ears of the same age only should be compared. Inasmuch as the appearance of the ear on the stalk had proved an unsafe criterion of age it was decided to tag each ear as the silk made its appearance. Every morning, therefore, between the hours of 9 and 12

throughout the flowering period each plot was carefully searched for all specimens showing the first appearance of silks, and tags bearing the respective dates were attached thereto. To facilitate the later gathering of sample ears of a given date tags of different colors were used on different days. This detail proved of great assistance in the rapid collection of samples. Careful records were kept of each day's tags so that the rate of silking and the length of the silking period of each variety were known, and data were at hand to tell when sufficient material was available for the various tests.

Samples were taken for canning tests at the ages of 15, 20, 25, and 30 days, and samples for chemical analysis were taken at the ages of 5, 10, 15, 20, 25, and 30 days.

Since the behavior of varieties and the chemical composition of samples is affected by climatic factors data upon temperature and rainfall are given in the accompanying chart which shows the conditions under which the corns were grown. Complete temperature records covering the test period were not obtainable at the Arlington experiment farm as only maximum and minimum daily temperatures were recorded. The figures given are those obtained from the United States Weather Bureau at Washington whose station is situated but a short distance across the river from the Arlington farm. Careful comparison of the records from the two sources have shown that differences in temperature in the two places were too small to be of significance. In most cases the temperatures were practically identical, and the data given, therefore, may be considered as fairly representing the actual temperature conditions under which the corn was grown. The data upon rainfall were obtained at the Arlington farm.

Examination of the chart shows that the mean daily temperature for the growing and maturing period of the corns under study was somewhat above the 49-year average during May and June, but fairly close to it during July. August was somewhat below the average.

Taken as a whole, the season was rather wet, there being an excess of  $3\frac{1}{4}$  inches of rainfall for the months May to August, inclusive. The excessively heavy downpour of September 2, while occurring before all the samples were harvested, had little if any effect upon those remaining to be handled since they were well along toward maturity at this time. It will be noted that the rainfall was well distributed throughout the test period. While several rather heavy rains occurred, in no case was there a drowning of the corn as the underground drainage system rapidly removed all excess of moisture.

It is regretted that data upon soil temperatures and moisture content of the test plots are not available for presentation in this connection.

## EXPERIMENTAL RESULTS

### THE SILKING PERIOD

The significance of the silking period of corn as a practical aid in determining when and how rapidly a field of corn would come to canning maturity seems never to have been appreciated, but the fact that the quality of the canned product is intimately associated with the stage of ripeness of the corn when it goes into the can makes the consideration of this matter of importance. Burrill and McCluer (12, 13) in studies upon a considerable number of varieties noted the date of the first bloom, the date of the full bloom and that when the corn was first fit for use. Butz and Baldwin (16) recorded the date of tassel and the dates of the first and last ears upon a number of varieties including Crosby, Mammoth Sugar, and Stowell's Evergreen. Munson and Gould (42) observed the dates of the first appearance of tassels, the appearance of silks and of edible maturity upon 15 varieties of sweet corn, and Gould (26, 27) gave like data for the two succeeding years upon 25 varieties. Butz (15) recorded the date of tassel and the date of the first and of the last ears. Kiesselbach (34) in investigations upon field corn gave attention to the length of the period of pollination, the life of the pollen and the life of the silk; and in the tabulation of results of his experiments has shown the dates of tasselling along with the dates when the corns were ripe.

The methods followed in the collection of data upon the silking period in the present investigations made it possible to study this subject in a more comprehensive manner than has been hitherto attempted, and inasmuch as these data show the relative earliness of the different sweet corns under study, the rate at which each of the test plots came into flower, the length of the silking periods for the different corns, to some extent also the relation of meteorological factors to the silking period, and various other factors, they have been arranged in graphic form in Figure 2 which is presented herewith.

An error of minor importance in this chart exists with respect to relative earliness in the case of Golden Bantam, Crosby, Charlevoix, and Howling Mob which, as stated before, were planted on May 17, one week earlier than all the others. These graphs are based upon the actual dates of appearance of silks regardless of the date of planting; but Latta (36) has shown that in Indiana a difference of one week in planting at this season makes a difference of about 5 days in the time of maturing, that planted earlier requiring longer to mature. The amount of correction to apply for this factor in the present case is impossible to determine accurately as no check was secured upon this point. The change in relative positions of these varieties would be small, in any case making them but one or possibly two days later than indicated on the graphs.

It will be seen that even after taking into consideration the correction just mentioned, Golden Bantam was the earliest of all the varieties tested, the first silks appearing on July 3 and the maximum silking being reached just one week later. Howling Mob was earliest of the whites. It first showed silks on July 8, and the high point of its silking period was 5 days later. Vaughn's Bantam Evergreen was the latest of the yellow varieties, its first silks appearing on July 14. Maximum silking in this variety took place about 10 days later. The Evergreens and Country Gentleman were the latest of the whites. The latter which began silking one day later than Stowell's Evergreen showed its first silks on July 21, and on July 28 the highest number were recorded. Both varieties of Dent Corn were later than any of the sweets, and Boone County White considerably so. It began silking on July 30 and reached its maximum about one week later.

In the presentation of data later on in this report it will be shown that the rate at which a field of corn comes into flower and the length of the silking period make it possible to estimate with considerable accuracy when the corn will be ready for canning and how long the canning season for that field is likely to be. Of special interest therefore, are these figures as illustrating this point. For example, it will be seen by the figures for Golden Bantam and Howling Mob that these plots came into silk very rapidly and the periods during which abundant silking occurred were brief. This indicates that the bulk of these corns would come to edible or canning maturity during a few days only and the canning season would be correspondingly short, which proved to be true. The figure for Potter's Excelsior, on the other hand, shows that the period during which abundant ear formation was taking place was much longer, and indicates that ears for table and canning purposes could be secured over a considerable period of time, which likewise was found to be the case.

A variety of factors may influence results of this sort and therefore too much importance should not be attached to a single set of experiments. The use of inferior seed, difference in cultural practices, meteorological conditions, and other factors may be responsible for certain variations in rate of silking and the length of the silking period, and it is possible that one or more of these were responsible for some of the irregularities showing in these graphs.

Attention is called particularly to the depression in the figures for the dates of July 15 and 27, respectively. Reference to the chart on temperature and rainfall will show that during the 24 hours preceding each of these dates there was a distinct drop in temperature, and in the case of the earlier date this fall in temperature was accompanied by heavy rainfall.

It is believed that comparable tests of this sort made in the different sweet-corn producing sections of the country would not only be of value to those in-

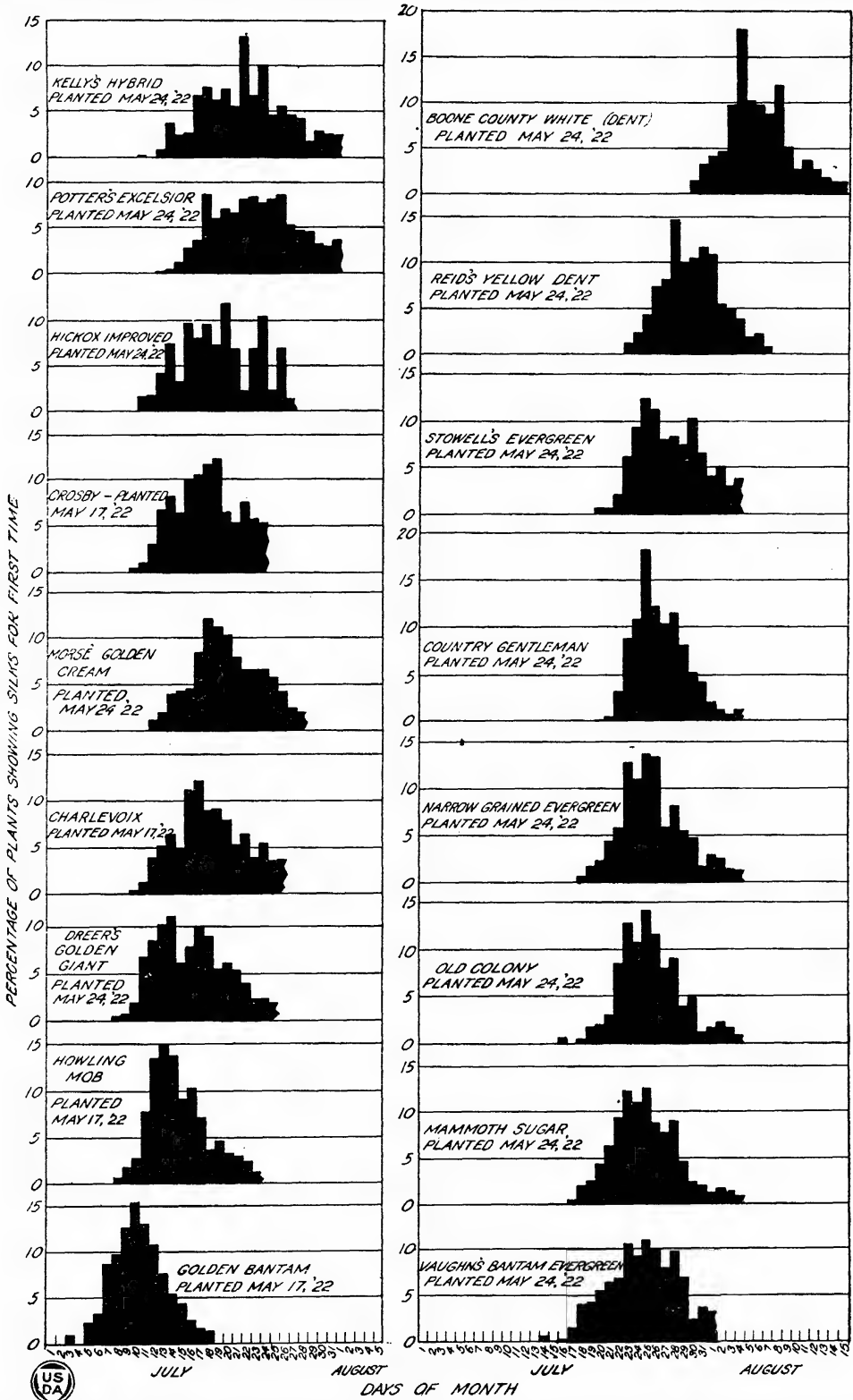


FIG. 2.—Graphs illustrating the silking period of 15 different varieties of sweet corn and 2 varieties of field corn used in the present studies

terested in the canning of the corn but also would throw light upon the relation of latitude, temperature, and other seasonal factors to the growing of the corn, and the selection of desirable varieties.

## DEVELOPMENT OF THE EAR

To the grower and the canner alike information upon the development of the ear, and especially of the kernel of the corn, is of great practical interest and importance. The grower looks toward the financial returns for his crop, which is usually determined directly by the weight of corn produced, and the canner is desirous of obtaining the raw corn at the stage of maturity which will give the highest yields consistent with high quality. In the present work, therefore, an attempt was made to get some information upon this point which would in a measure serve as a guide in regular practices. Accordingly, representative ears of known age were selected from each variety beginning 5 days after the first appearance of silk and samples taken at age intervals of 5 days throughout the growing and maturing season. These were removed at once to the laboratory where weights were recorded upon the freshly plucked ears, the husked corn, cut-off kernels, cob, and husks.

So far as possible the ears were dressed for weighing to conform to practical working conditions, weights being taken after excessively long ear stalks had been broken to normal length, etc. In removing the kernels for weighing great care was exercised to remove as nearly as possible the entire kernel without including any of the cob. For this purpose sharp pointed knives were employed and only one row of kernels cut at a time. In the case of very young corn the kernels were merely pushed or shelled out with the tip of the blade without cutting at all. The accompanying table shows the results obtained.

TABLE I.—*Showing the increase in size of ears and changes in the proportion of husk, cob, and cut corn during the development of different varieties of sweet corn*

Variety and stage of maturity in days	Date tagged	Number of ears taken	Average weight of ear	Percent of cut corn	Percent of cob	Percent of husk	Average weight of husked ear	Per cent of cut corn from husked ear
<b>Golden Bantam:</b>								
5.....	July 8	10	91.8	7.8	17.5	74.6	23.3	30.7
10.....	July 7	15	152.0	12.2	34.0	53.7	70.3	26.3
15.....	July 6	11	155.4	25.9	31.9	42.1	90.0	44.7
20.....	do.....	9	176.1	27.4	29.6	42.9	100.5	48.0
25.....	July 7	8	187.5	46.6	21.0	32.3	126.9	68.9
30.....	July 11	6	194.1	48.5	21.8	29.6	136.6	68.8
<b>Howling Mob:</b>								
5.....	July 12	24	137.5	-----	-----	64.5	48.9	-----
10.....	July 8	5	284.0	-----	-----	52.8	134.0	-----
15.....	July 12	11	280.4	23.9	43.5	32.5	189.5	35.4
20.....	do.....	6	275.8	35.3	33.2	31.4	189.1	51.5
25.....	July 13	6	355.0	39.2	36.8	23.9	270.0	51.5
30.....	July 18	5	346.0	45.3	34.7	19.9	277.0	56.6
<b>Dreer's Golden Giant:</b>								
5.....	July 13	19	140.0	11.0	23.5	65.4	48.4	31.9
10.....	July 10	10	240.5	10.8	34.9	54.2	110.2	23.6
15.....	July 11	13	251.1	24.8	39.6	35.5	161.9	38.5
20.....	July 13	6	278.3	36.8	35.0	28.1	200.0	51.2
25.....	July 17	6	280.0	41.9	31.2	26.8	205.0	57.3
30.....	July 19	5	338.0	50.5	26.9	22.5	262.0	65.2
<b>Charlevoix:</b>								
5.....	July 12	24	141.3	-----	-----	79.3	29.1	-----
10.....	July 11	11	238.6	16.0	29.9	54.0	109.5	34.8
15.....	July 17	10	338.0	28.5	31.8	39.6	204.0	47.3
20.....	July 14	6	339.1	36.1	33.1	30.7	235.0	52.1
25.....	July 18	7	408.5	46.6	25.7	27.6	295.7	64.4
30.....	July 15	6	345.8	49.8	24.6	25.5	267.5	66.9
<b>Crosby:</b>								
5.....	July 13	18	113.0	15.9	21.6	62.4	42.5	42.2
10.....	July 11	6	180.9	22.4	32.8	44.7	100.0	40.6
15.....	July 12	12	192.9	21.5	42.6	35.8	123.7	33.7
20.....	July 14	6	255.0	37.2	29.4	33.3	170.0	55.8
25.....	July 21	8	212.5	47.0	31.2	21.7	166.2	60.0
30.....	July 19	4	227.5	47.8	26.3	25.8	168.7	64.4
<b>Morse's Golden Cream:</b>								
5.....	July 14	24	101.2	11.4	12.6	75.9	24.3	47.6
10.....	July 15	15	164.0	16.5	32.8	50.6	81.0	33.4
15.....	July 12	10	201.0	25.6	30.3	44.0	112.5	45.7
20.....	July 19	7	257.1	43.6	22.2	34.1	169.2	66.2
25.....	July 22	8	221.9	48.4	23.6	27.9	160.0	67.1
30.....	July 23	7	216.5	57.0	26.1	16.8	180.0	68.6

TABLE I.—Showing the increase in size of ears and changes in the proportion of husk, cob and cut corn during the development of different varieties of sweet corn—Continued.

Variety and stage of maturity in days	Date tagged	Number of ears taken	Average weight of ear	Percent of cut corn	Percent of cob	Percent of husk	Average weight of husked ear	Per cent of cut corn from husked ear
Hickox's Improved:	1922		Gm.				Gm.	
5.....	July 14	25	116.6	14.3	17.7	67.9	37.4	44.8
10.....	do	14	175.3	16.2	37.3	46.4	93.9	30.2
15.....	July 16	10	265.0	20.7	42.1	37.1	166.5	33.0
20.....	July 18	7	274.2			33.3	182.9	
25.....	July 20	6	310.0	36.5	32.3	31.1	213.3	53.1
30.....	July 22	5	243.0	41.1	41.2	17.6	200.0	50.0
Kelly's Hybrid:								
5.....	July 14	17	154.7	14.1	19.1	66.7	51.4	42.4
10.....	July 15	15	188.0	18.0	37.6	44.3	104.6	32.4
15.....	July 19	6	338.3	19.2	43.3	37.4	211.6	30.7
20.....	July 21	5	345.0	30.4	37.9	31.6	236.0	44.4
25.....	July 27	6	420.9	44.9	32.8	22.2	327.5	57.7
30.....	July 24	6	315.0	48.9	31.7	19.3	254.1	60.6
Potter's Excelsior:								
5.....	July 16	12	159.1	13.7	14.3	71.9	44.5	49.1
10.....	July 17	12	227.9	17.7	42.9	39.3	138.3	29.9
15.....	July 18	6	339.1	25.3	37.8	36.8	214.1	40.0
20.....	July 21	6	352.5	28.6	34.0	37.3	220.8	45.6
25.....	July 24	5	345.0	45.2	30.7	24.0	262.0	59.5
30.....	July 26	6	376.6	46.9	30.5	22.5	291.6	60.5
Vaughn's Bantam Evergreen:								
5.....	July 19	21	143.3	16.3	19.5	64.1	51.1	45.5
10.....	July 21	10	240.0	16.4	37.0	46.5	128.5	30.7
15.....	July 20	6	290.0	29.3	36.5	34.1	190.9	44.5
20.....	July 22	6	339.1	39.3	29.4	31.2	233.3	57.1
25.....	July 24	6	327.5	50.8	21.9	27.2	238.3	69.9
30.....	July 26	6	400.0	55.0	24.5	20.4	318.3	69.1
Mammoth Sugar:								
5.....	July 19	18	148.0	19.8	23.4	56.7	64.1	45.8
10.....	July 21	10	379.0	13.4	40.8	45.7	206.0	24.7
15.....	July 24	6	465.0	21.6	41.7	36.6	295.0	34.1
20.....	July 23	6	508.3	30.0	34.5	35.4	328.3	46.4
25.....	July 25	6	441.6	43.5	31.5	24.9	331.6	56.0
30.....	July 27	5	498.0	49.0	28.1	22.8	384.0	63.5
Old Colony:								
5.....	July 20	14	163.5	19.6	21.8	58.5	67.9	47.3
10.....	July 19	9	251.6	16.5	39.5	43.9	141.1	29.5
15.....	Aug. 3	5	272.0	28.3	37.8	33.8	180.0	42.7
20.....	July 22	6	340.0	39.9	30.1	29.9	238.3	56.9
25.....	July 23	6	420.0	51.1	26.8	22.0	327.5	65.6
30.....	July 25	6	410.9	58.2	25.5	16.2	344.1	69.4
Narrow Grained Evergreen:								
5.....	July 20	15	164.3	18.5	22.4	59.0	67.3	45.2
10.....	July 21	11	228.1	18.9	41.7	39.3	138.1	31.2
15.....	July 25	7	394.2	27.7	40.5	31.7	269.2	40.5
20.....	July 23	6	340.0	43.6	33.0	23.3	260.9	56.8
25.....	July 28	6	494.1	54.4	23.9	21.6	387.5	69.4
30.....	July 22	6	435.0	59.9	22.4	17.6	358.3	72.7
Country Gentleman:								
5.....	July 22	27	103.1	20.6	21.5	57.8	43.5	48.9
10.....	July 23	12	191.6	21.9	38.0	40.0	115.0	36.5
15.....	July 25	10	216.0	29.4	41.4	29.1	153.0	41.5
20.....	July 26	10	253.5	46.1	29.6	24.2	192.0	60.9
25.....	July 23	10	257.0	55.8	26.4	17.7	211.5	67.8
30.....	July 24	10	216.5	60.7	24.0	15.2	183.5	71.6
Stowell's Evergreen:								
5.....	July 23	17	216.4	15.3	29.7	54.9	97.6	34.0
10.....	July 22	10	328.5	18.8	40.2	40.9	194.0	31.9
15.....	July 25	12	327.5	28.7	44.0	27.2	238.3	39.5
20.....	July 26	6	411.6	45.9	31.8	22.2	320.0	59.1
25.....	July 27	6	418.3	52.1	24.7	23.1	321.6	67.8
30.....	July 29	5	474.0	62.8	23.6	13.5	410.0	72.6
Reid's Yellow Dent:								
5.....	July 28	10	279.0	12.3	22.7	64.9	98.0	35.2
10.....	July 24	8	333.6	15.1	31.6	53.2	156.2	32.4
15.....	July 30	6	394.1	23.6	48.8	27.5	285.9	32.6
20.....	July 28	6	485.8	43.5	37.9	18.5	395.9	53.4
24.....	July 31	6	485.8	50.5	32.8	16.6	405.0	60.4
30.....	July 29	4	580.0	57.3	23.2	19.4	467.5	71.1
Boone County White:								
5.....	Aug. 3	10	189.0	10.3	20.9	68.7	59.0	33.0
9.....	Aug. 1	8	295.6	12.6	39.1	48.2	153.1	24.4
15.....	Aug. 8	6	405.0	18.7	41.5	39.7	244.1	31.0
20.....	Aug. 4	6	478.3	28.9	39.0	32.0	325.0	42.5
25.....	Aug. 5	4	520.0	38.2	32.2	29.5	366.2	54.2
30.....	Aug. 6	6	503.3	44.7	28.6	26.6	369.1	60.9



The outstanding feature of this table is the illustration of the fact that for the ear as a whole there is a progressive gain in weight throughout the growing period; that as a rule the cob shows its highest percentage weight at about 15 days from the first appearance of silks, after which it falls off; and that in general the kernels continue to increase in weight throughout the whole period, up to 30 days of age. This last fact is of particular importance as showing that if the highest quality of canned corn is to be produced the proper maturity must be considered as of more importance than highest yield.

It is indicated by these figures that under the conditions of the present experiment the heaviest yields of cut corn per ton of ears was produced by Country Gentleman and Stowell's Evergreen. Others which stood well from this standpoint were Morse's Golden Cream, Narrow Grained Evergreen, and Old Colony. Of the earlier corns, Crosby, Dreer's Golden Giant, and Charlevoix gave fair cuts, though these were considerably below Country Gentleman. The production from few-rowed varieties and from those having a disproportionately large ear was low.

Objection may be made to this table on the ground that too few ears were taken to make the figures truly representative of average conditions. The use of small numbers of ears was made necessary by the circumstances under which the work was done, but in the selection of ears for these tests an earnest endeavor was made to choose those ears which were truly representative of the whole and of the variety under study. While, therefore, slight irregularities may be noted in the progress of changes as indicated by these figures, it is believed that, taken as a whole, they give a fairly close approximation of actual average conditions.

#### TOUGHNESS OF KERNEL

Although flavor and consistency, or body, are important factors in determining the quality of canned corn, more and more it is coming to be realized that the tenderness or toughness of the kernel is of even greater importance. Immaturity is sometimes masked in the canned corn, in part at least, by the addition of starch, and too heavy consistency due to the use of overmature corn is often avoided by the use of a greater proportion of liquor; but general toughness can not be masked, and it is this more than anything else which unfavorably affects the quality of canned corn. This point has been emphasized by Burton (14), who states that "commercial grades of canned Country Gentleman corn, as far as they are concerned by maturity, differ only in the proportion of tough and tender kernels present."

Burton (14) undertook a study of the cause of increased toughness in overmature corn and sought a practical means of determining maturity. He reported that the hull of Country Gentleman corn did not thicken with increasing maturity, but that it did lose progressively in moisture. The crude fiber figure for the hulls alone was found to be nearly constant at all maturities. The results of his experiments indicated that the specific gravity of the kernels afforded a means of differentiation of old from young corn.

Remington (49) in his report of analyses of the canned product from Crosby, Country Gentleman, Evergreen, and Golden Bantam varieties recorded slight differences in the percentage of crude fiber in the varieties studied, and stated that in his experience the amount of fiber tended to increase with the maturity of the corn. From the data presented, however, it is impossible to judge of the amount of this tendency as the analyses were made of material secured from different sources and apparently without information as to the exact age of the corn when it was canned.

During the last few years a number of workers have made use of mechanical appliances in studies upon the maturity of various fruits and vegetables, the idea being based upon the fact that with increasing maturity alterations in the tissues occur which affect their resistance to pressure or penetration. Thus Rosenbaum and Sando (52) made use of a modified Joly balance equipped with a puncturing needle in studying the resistance of tomatoes to penetration, and Lewis, Murneek, and Cate (37) and Murneek (43) employed a mechanical puncturing device to determine the ripeness of pears. Rudnick and Bakke (53)

likewise made use of this idea in the study of the resistance to puncture of the pericarp of sweet corn as related to maturity and other factors, and reported an increase in the resistance of the pericarp to puncture with increasing maturity of the corn.

In laying out the plans for the present investigations it seemed to the writers that inasmuch as the toughness of the kernel is of great importance in determining the quality of the canned product from sweet corn a wider application of the puncture test could be made to advantage and that a more convenient and practical instrument could be made than had hitherto been employed. A simple direct-reading puncturing apparatus was therefore devised, and tests were conducted upon the different varieties of corn at 5-day intervals throughout the critical period. A diagram showing the features of this instrument is presented herewith.

This apparatus consists of a glass tube 13 inches long, having an internal diameter of  $\frac{3}{4}$  inch and a plunger constructed of aluminum rod  $\frac{3}{16}$  inch in diameter and 14 inches long, which is fitted with a small needle holder at the outer end, a thin square metal indicator at the other, and attached just below this indicator to a steel wire coil spring. This spring is made from No. 22 steel piano wire so coiled as to allow of extension and contraction without coming into contact with either the inner walls of the glass tube or the plunger passing through it at the center, and is attached on one end to the upper end of the plunger, as already described, and on the other at the lower end of the glass tube. The plunger is kept properly centered by means of the square metal indicator tip at the upper end, which moves sufficiently freely to make friction insignificant, and by a thin smooth metal guide at the bottom of the tube, which allows of free movement with minimum friction.<sup>4</sup> The needle consists of a piece of No. 16 brass wire,

<sup>4</sup> Numerous trials were required to secure the proper length and extension of the spring. It was essential that the spring be so constructed and adjusted as to operate satisfactorily without fatigue within the effective range of the tube, and to meet the needs as regarded the resistance of the kernels to puncture. It was likewise necessary that the spring be sufficiently sensitive to make the readings of resistance clear-cut and accurate. This condition was satisfactorily attained in the apparatus described.

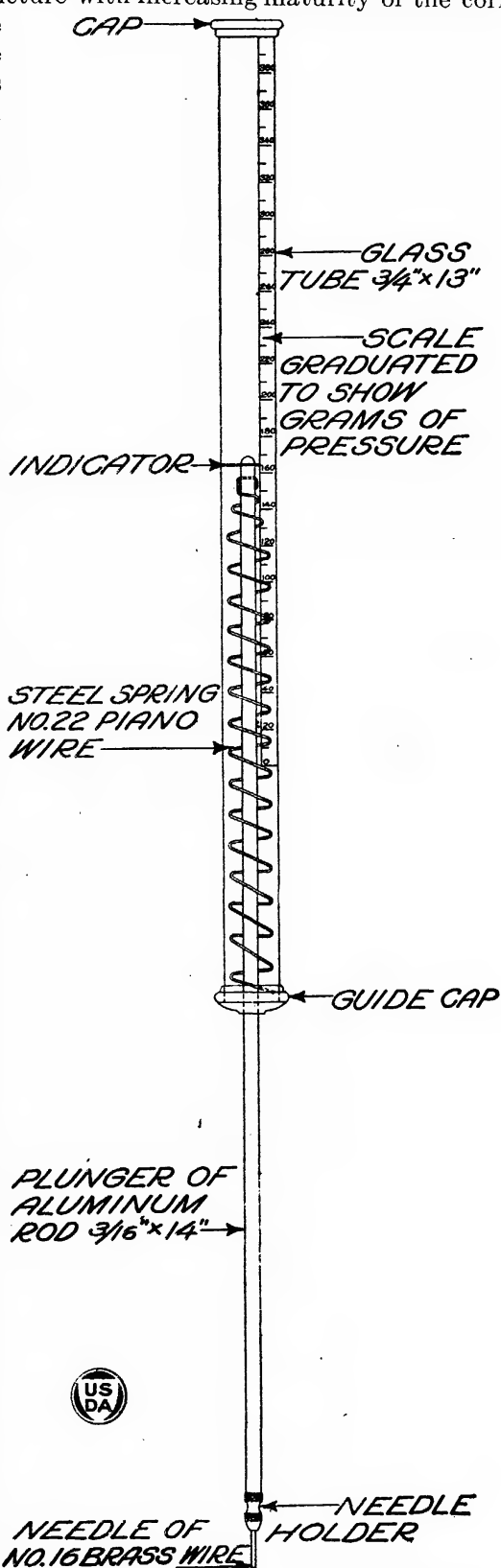


FIG. 3.—Diagram of instrument used in making puncture tests

three-fourths inch long, the tip of which is carefully squared to right angles with the axis.

The instrument thus constructed is carefully standardized and a graduated scale reading in grams pressure equivalents placed upon the glass tube. The zero point is determined by the position of the indicator when the plunger comes to rest while hanging freely in a vertical position. Other points are determined by the position of the indicator when balancing the proper corresponding weights.

The resistance of the kernels to puncture is determined by clear-cut positive readings, for when the pericarp is penetrated there is a sharp reaction of the spring. Definite measures of toughness of kernels on different parts of the ear are rapidly made. The instrument is easily operated by the right or left hand, as desired, and the ear of corn may be held either in the other hand or placed upon a table for the test.

This instrument was in daily use throughout the season both in the laboratory and in the field, and its efficiency was found unimpaired at the conclusion of the work.

Puncture tests were begun with the different varieties when the ears had reached the age of 10 days from the first appearance of silk and were made thereafter at 5-day intervals up to 30 days. From 3 to 5 representative ears were tested at each stage and 30 to 40 kernels on each ear, including those situated at the tip and butt as well as at the middle of the ear. The kernels tested were uniformly distributed over the whole ear though occasionally all the kernels of a single row were tested. The results of an average test are illustrated by the following table, which shows the readings obtained upon 4 ears of Howling Mob at the age of 15 days. The figures represent grams of pressure exerted by the needle of the instrument upon the kernels before the pericarp was penetrated.

TABLE II.—*Results of an average puncture test upon 4 ears of Howling Mob sweet corn at 15 days from date of first appearance of silks on these ears*

[Figures represent pressure in grams required before the needle penetrated the pericarp of the kernels.  
Date of test, July 27, 1922]

First ear	Second ear	Third ear	Fourth ear
220	215	210	200
220	235	220	180
240	205	250	180
230	185	230	220
180	195	225	245
230	230	230	220
240	175	210	200
250	230	260	195
210	225	270	195
210	225	185	160
200	220	225	200
230	240	220	190
235	215	250	210
180	175	230	190
220	195	240	210
280	260	220	180
200	255	255	220
200	240	235	210
200	210	270	205
220	225	200	220
210	185	235	205
240	220	200	195
220	210	210	200
225	210	215	180
270	210	250	180
220	230	275	230
210	230	220	195
255	180	230	235
235	205	215	205
200	195	190	180
210	-----	-----	210
190	-----	-----	205
Av. 221	214	230	201

Grand average, 216.

These results are typical of those obtained with all varieties, though in some instances greater differences in the range of toughness of individual kernels were observed, as well as, occasionally, greater differences in the averages for the test ears. On the whole, the results of these tests showed clear-cut and significant differences in the toughness of the kernels at different stages of maturity, as will be shown by the curves which follow shortly.

What was at first a surprising result in the test upon individual ears was the finding that the kernels at the butt of the ear as well as at the tip were distinctly more tender than those nearer the middle. This was found to be almost universally true, and in searching for the explanation it was thought that these differences were probably due to differences in age of the kernels as determined by the time of pollination of the silks. This idea was confirmed in conversation with Dr. C. P. Hartley, of the Office of Cereal Investigations, who has demonstrated that the silks from the middle portion of the ear are the first to appear and hence are first to become pollinated.

With the increase in maturity there was found to be a progressive increase in the resistance of the kernels to puncture. This will be shown by the series of curves of the different varieties of sweet corn tested which are presented herewith. As is to be expected of curves based upon the findings from but relatively few ears, irregularities in form are observed. These of course would be absent in curves based upon a larger number of tests.

Little comment upon these curves is necessary. Their outstanding feature is the pronounced and rather rapid rise indicating increasing toughness of the kernels. In no case did corn remain tender for any considerable period, though a tendency in this direction is shown in the case of Golden Bantam. Some varieties are distinctly tougher than others even while immature, as is shown, for instance, in the case of Crosby. (See fig. 4.) This slightly greater toughness of the Crosby, in some strains at least, was also observed in field studies in Maine during the 1922 canning season in which ears of about the same degree of maturity, as indicated by the thumb-nail test for the condition of starch, gave a little higher reading than was considered normal for most varieties at that stage grown at Arlington farm. Extreme toughness was attained by Country Gentleman (see fig. 6) and Kelly's Hybrid (see fig. 5) during the later stages, while several others, as, for instance, Golden Bantam, Dreer's Golden Giant, and Charlevoix (see fig. 4), did not approach an extremely high mark even at the end of 30 days. While these findings can not be analyzed in too great detail, being based upon the results of one season's work only and in a restricted area, they do show significant tendencies which deserve more careful study. It may be of interest to note the relation of toughness as indicated by these tests to corns in prime condition for canning. Conclusions drawn from these laboratory tests were surprisingly closely confirmed by a number of trained canning factory superintendents who were asked, during visits to various plants in Maine, to select ears from cannery stock representing corn in prime canning condition, ears that were immature, and those that were over mature. These combined to place the critical period, when corn was in prime condition, at the stage in which readings from 250 to 300 grams were obtained by use of the puncturing instrument. The reading of 325 grams was found very close to the "danger line" except in the case of some strains of Crosby mentioned above, which often were found to be still in the milk stage when this reading was obtained. Tentative conclusions based upon laboratory tests at Arlington experiment farm, Virginia, had already placed the readings of 250 and 325 as limits to the period during which the different varieties were in best canning condition.

It has not been possible in the present work to study fully the relation of temperature and other climatic factors to the increase in toughness of corn during

growth. Observations made in Virginia and Maine, however, indicate that in general no significant differences exist in toughness of corns which have reached canning maturity under the two sets of climatic and seasonal conditions. Apparent differences due to inherent characteristics of strains which have been touched upon above are considered as apart from this particular phase of the subject.

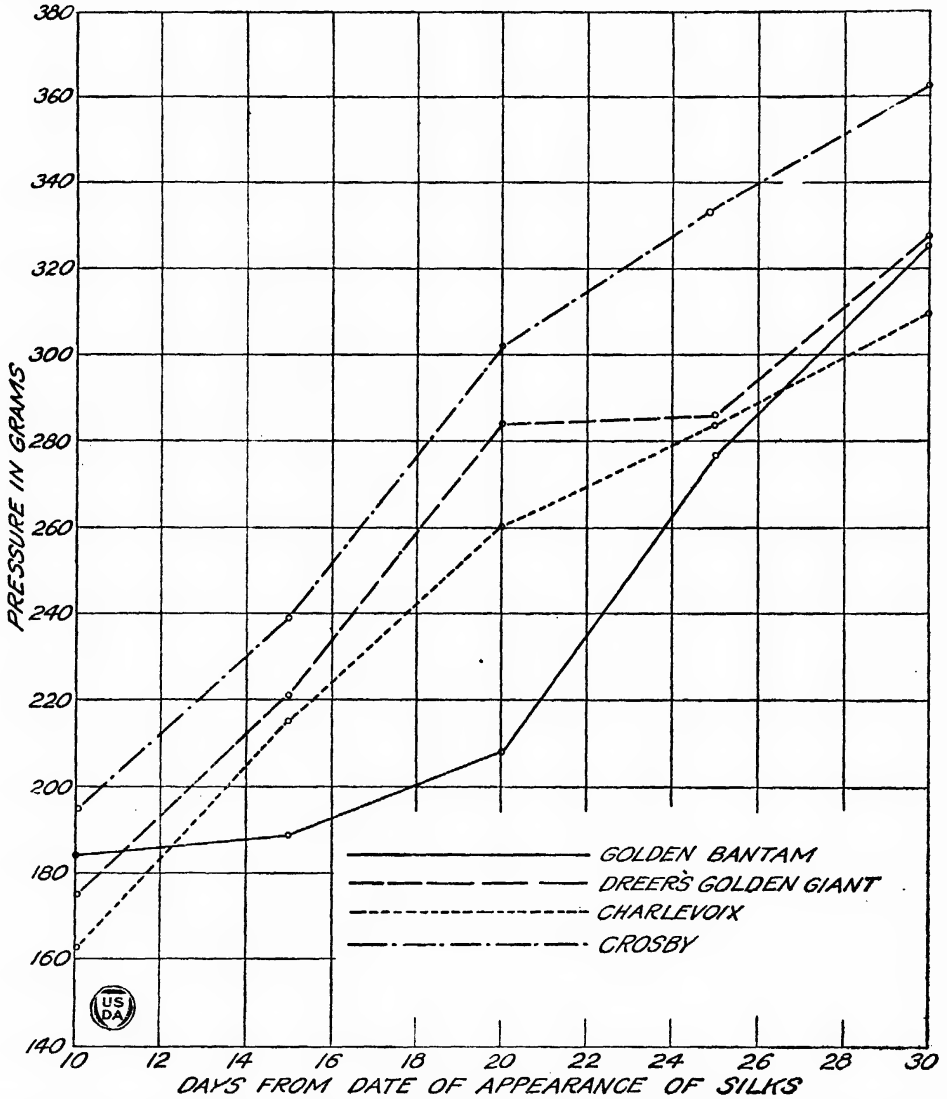


FIG. 4.—Curves illustrating the increase in toughness of kernels of Golden Bantam, Dreer's Golden Giant, Charlevoix, and Crosby sweet corns with increase of maturity

### CHEMICAL STUDIES

During the growth and development of the ear of corn the products of metabolism are transferred to the kernel where they are utilized in part in tissue building and in part are laid down as storage materials. The transformations undergone in these processes and the nature and amount of these changes account for the differences in quality of corns at various stages of maturity. As concerned in these activities a number of organic substances may be mentioned such as crude fiber and other carbohydrates, fats and allied substances, proteins, and those substances which give characteristic flavor, the chemical nature of which is not known.

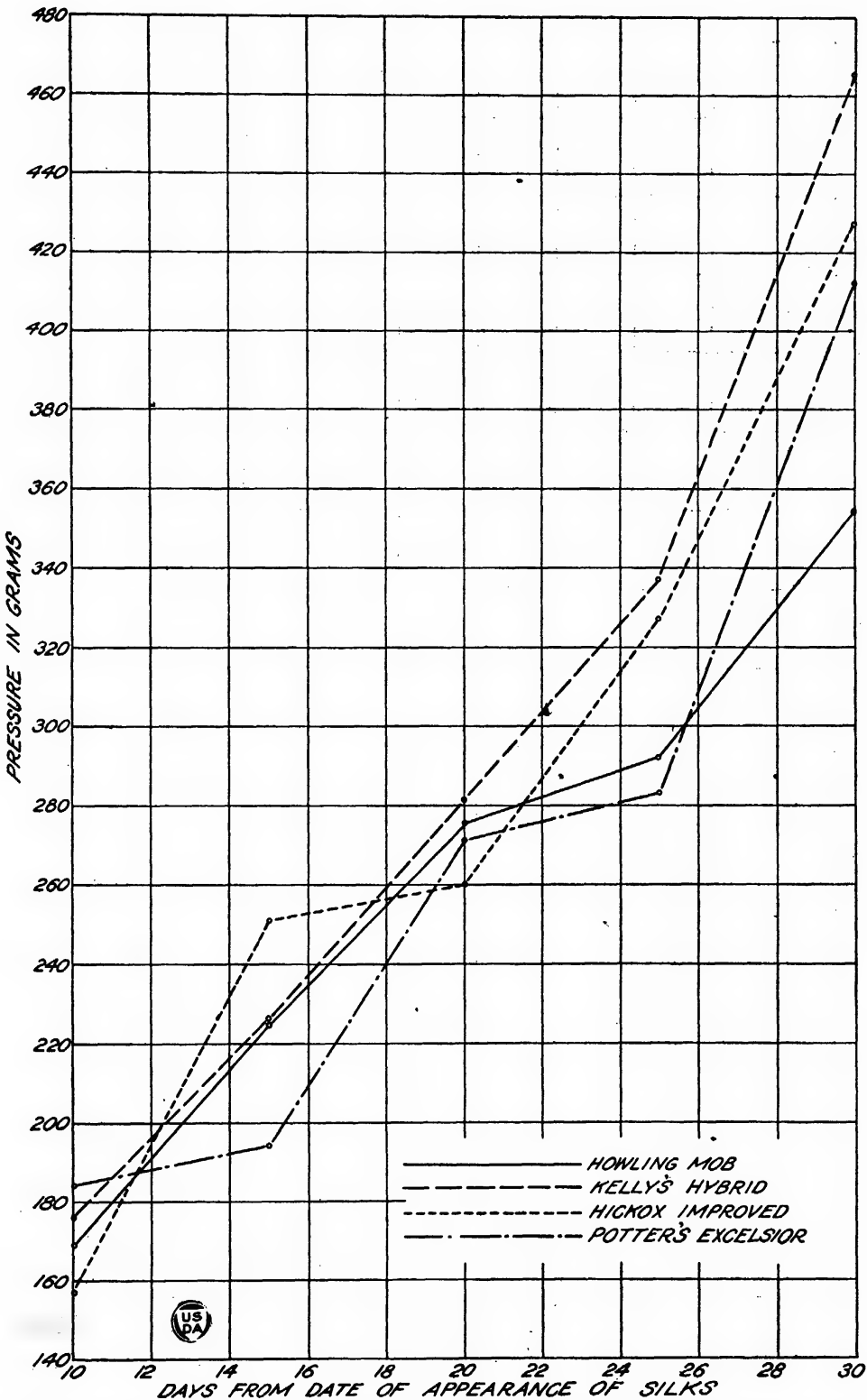


FIG. 5.—Curves illustrating the increase in toughness of kernels of Howling Mob, Kelly's Hybrid, Hickox's Improved, and Potter's Excelsior sweet corns with the increase of maturity

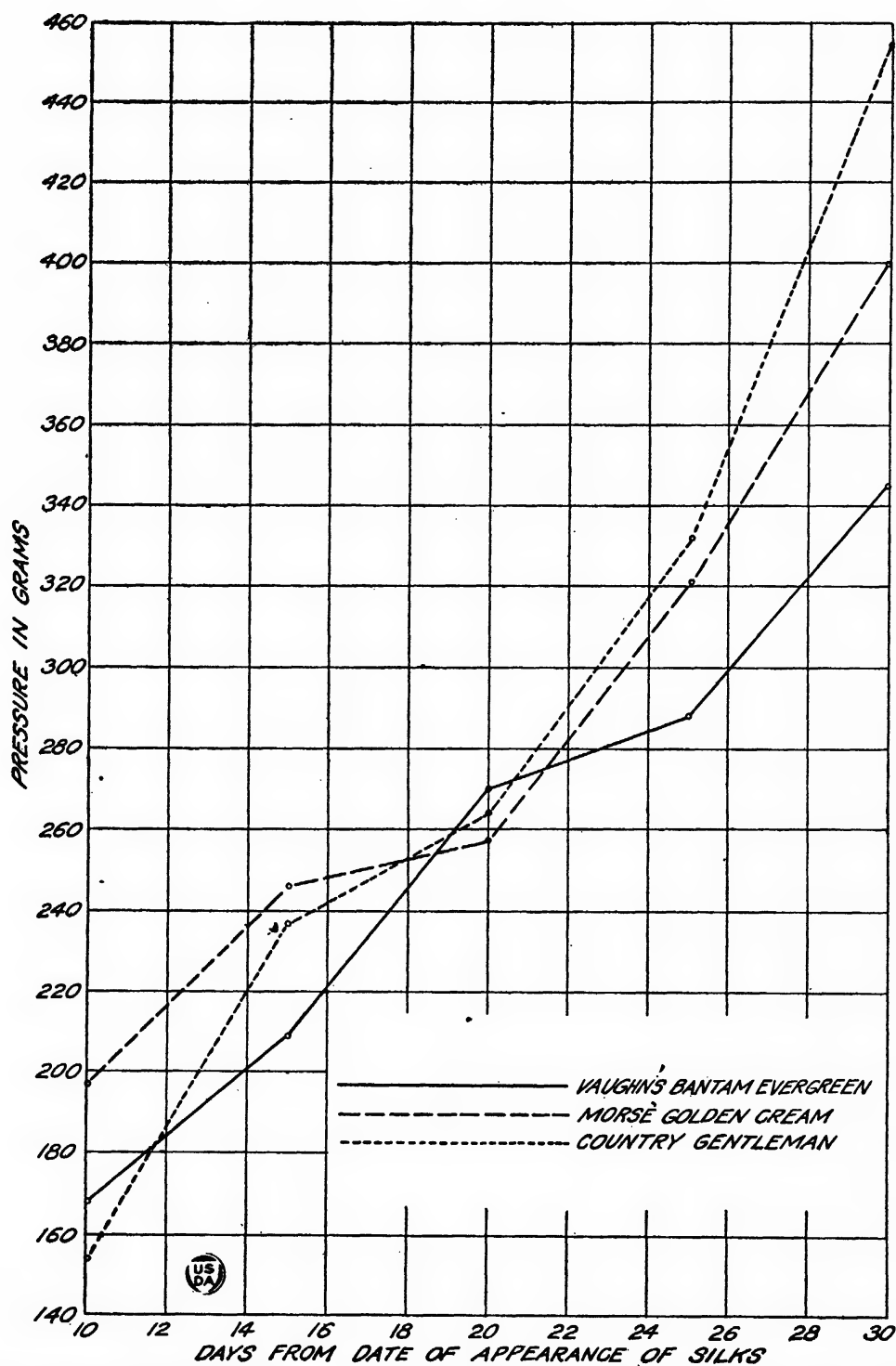


FIG. 6.—Curves illustrating the increase in toughness of kernels of Vaughn's Bantam Evergreen, Morse's Golden Cream, and Country Gentleman sweet corns with the increase of maturity

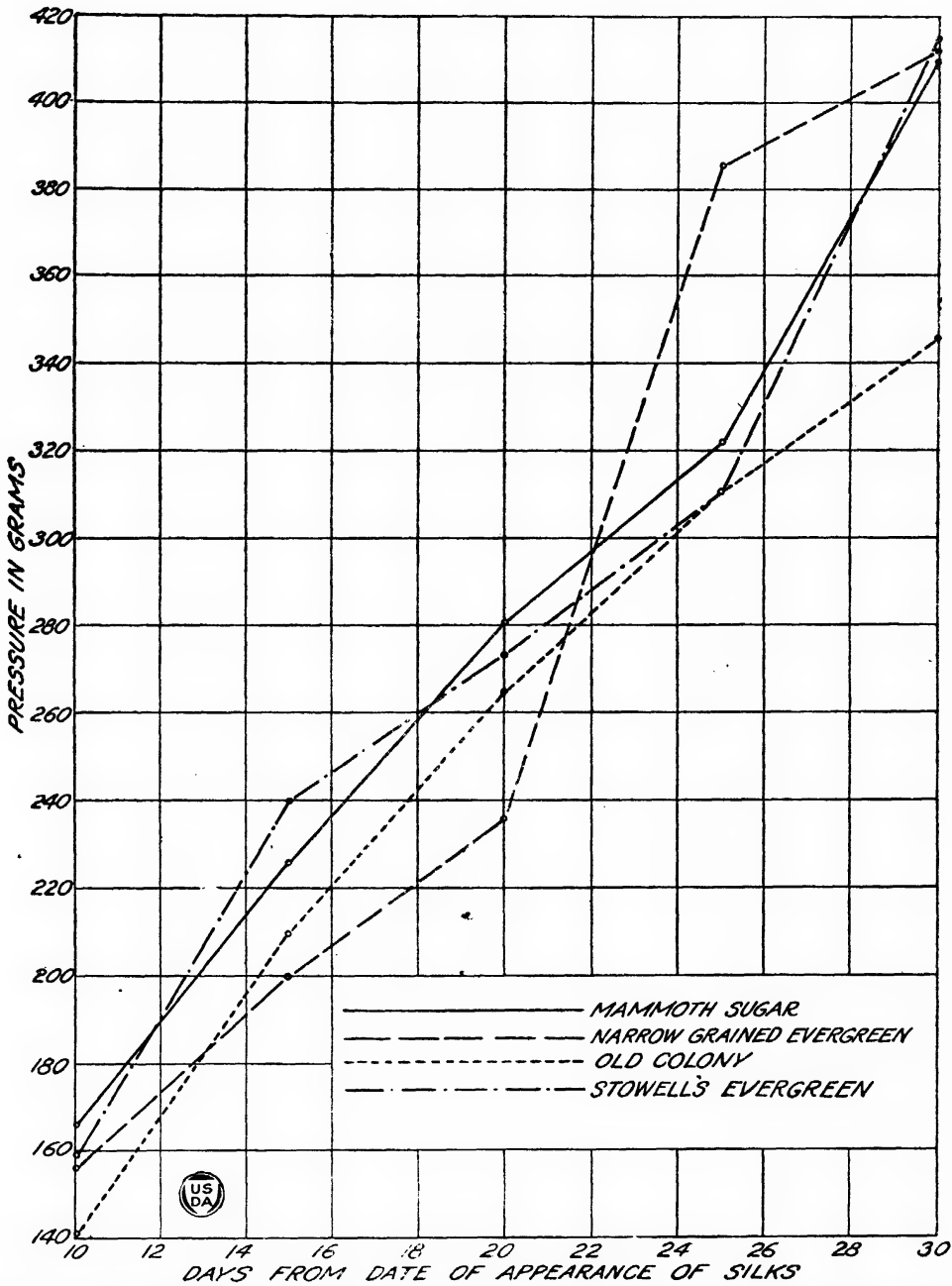


FIG. 7.—Curves illustrating the increase in toughness of kernels of Mammoth Sugar, Narrow-grained Evergreen, Old Colony, and Stowell's Evergreen sweet corns with the increase in maturity



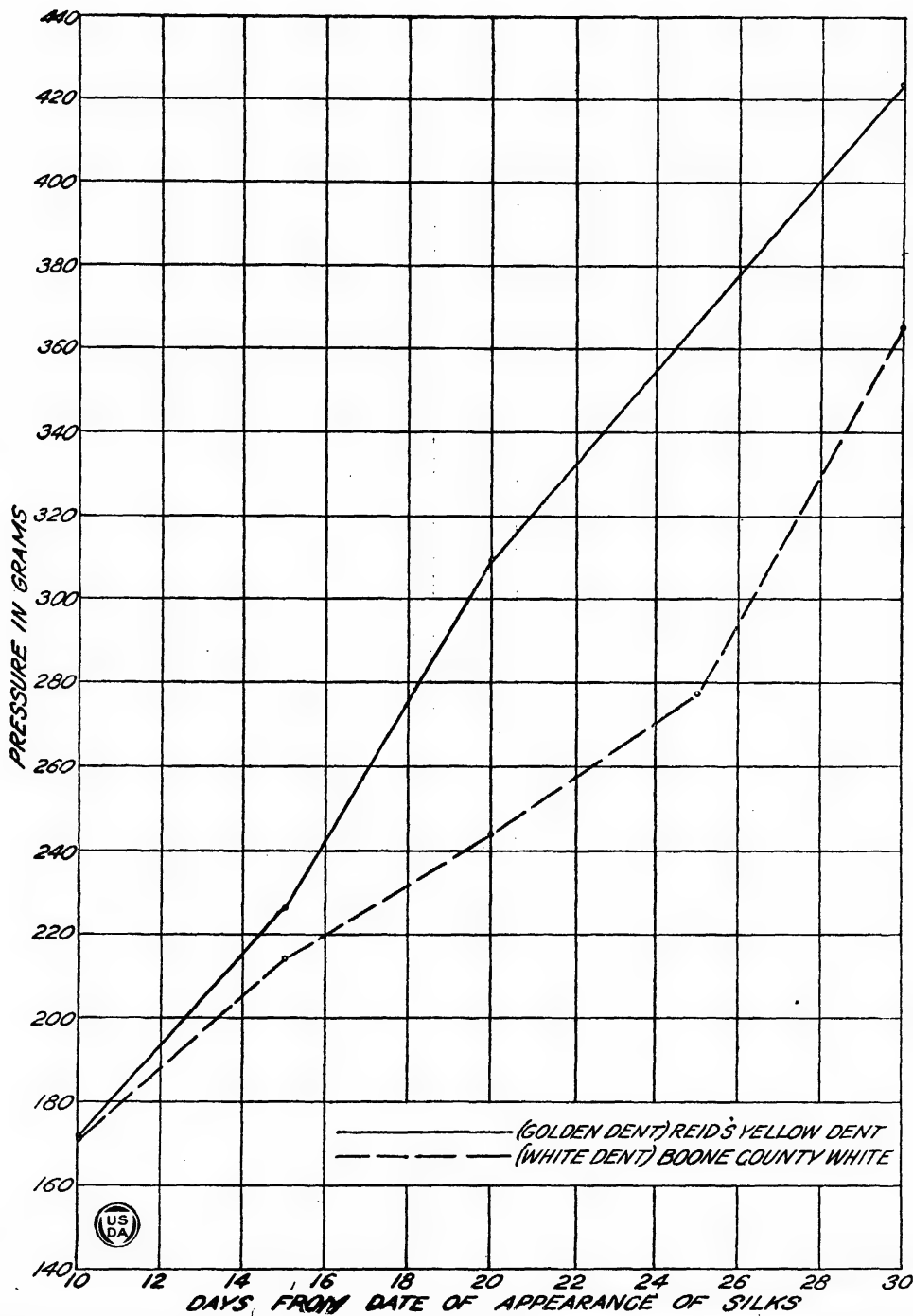


FIG. 8.—Curves illustrating the increase in toughness of kernels of Reid's Yellow Dent and Boone County White field corns with the increase of maturity

Much work has been done upon the chemistry of the developing maize plant, and the composition of the mature grains of sweet corn has been studied by a considerable number of workers. A much smaller number have given attention to the chemical transformations taking place during the growth and development of the kernels. The work of Salisbury (54), Atwater (5), Richardson (50, 51) and others has shown that the mature kernels of sweet corn have approximately the following chemical composition: Carbohydrates, 65 to 75 per cent; fats, 6 to 9 per cent; and proteins, 10 to 12 per cent. Appleman and Eaton (3), who have studied the chemical changes taking place in the developing sweet corn kernel, found that there was a progressive decrease in total sugars, an increase in fat, and a very great increase in starch. The crude fiber and proteins decreased. The exact significance of their figures from the standpoint of the fresh corn can not be determined, however, as their figures represented percentages on the basis of dry weight, and no figures upon moisture were given. These authors likewise studied early and late crops of sweet corn with reference to changes in the percentage composition of the fresh corn in equal lengths of time. In the early crop there was a progressive decrease in total sugars throughout the test period and the increase in starch, while progressive, was much slower than in the late crop. These findings were reported on the basis of the fresh green weight of corn. In a later paper Appleman (1), in his study of the reliability of the nail-test for predicting the chemical composition of green sweet corn, recorded a progressive decrease in sugars throughout the premilk, early dough, and dough stages, and an increase in starch. The same held true for both early and late crops of Stowell's Evergreen.<sup>5</sup>

Since the carbohydrates constitute such a large portion of the sweet corn kernel and seem so important as affecting the quality of the canned product the present chemical investigations have been confined to a study of the nature of and the changes in this group of substances.

#### METHODS OF ANALYSIS

The kernels were cut from 5 to 20 ears (the number depending upon the degree of maturity) of freshly plucked corn of known age, the material was thoroughly mixed and 100 gram samples, taken in duplicate, were carefully weighed out and transferred to flasks.<sup>6</sup> Immediately after weighing, 95 per cent alcohol was added in sufficient quantity to make the alcohol content of the sample 70 to 80 per cent. The samples were brought nearly to the boiling point of the alcohol and then sent away. The sampling was concluded within 3 to 4 hours from the time the ears were taken from the stalks. Canning samples were prepared at the same time.

After standing for some days each sample was handled in the following manner: The alcohol was decanted through an extraction thimble, more alcohol added to the corn, thoroughly shaken and again decanted. This was repeated several times. Finally the residue was transferred to the extraction thimble and thoroughly extracted with alcohol in the Soxhlet apparatus. In this way the greater part of the extraction was done in the cold, thus minimizing the possibility of alteration of the sugars by heating. Extraction of the residue in the Soxhlet apparatus assured the removal of the last traces of sugar. The different portions of extract from a given sample were united and then brought to volume.

Aliquot portions of this extract were examined for sugars, determinations being made both before and after inversion with hydrochloric acid, according to the methods of the Association of Official Agricultural Chemists (4). The

<sup>5</sup> Percentages calculated on basis of dry weight.

<sup>6</sup> In the 5 and 10-day samples it was impossible to prevent the inclusion of a small amount of chaffy material from the cob. There was very little, however, in the other samples.

residue was used for the determination of polysaccharides. Total polysaccharides were determined by inversion with hydrochloric acid, estimation of sugars and calculation as starch. To determine the polysaccharides soluble in cold water 1 gram of the alcohol-insoluble residue was ground in a mortar with 40 to 60 c. c. of distilled water, allowed to stand for several minutes, the milky supernatant portion decanted, and the process repeated several times. Finally the whole was washed into a 250 c. c. volumetric flask and brought to volume with distilled water. The sample thus prepared was allowed to stand over night. The milky supernatant portion was then decanted, mixed with a small amount of dry infusorial earth and then filtered by means of suction through a dry asbestos mat filter into a dry flask. Aliquot portions of this were transferred to boiling flasks, inverted with hydrochloric acid and the sugars determined in the usual way. The cold-water-soluble polysaccharides were calculated as dextrin. Table III shows the results of the analytical work.

TABLE III.—*Showing results of chemical analyses of 17 varieties of corn sampled at intervals of 5 days throughout the whole growth period*

Variety and age in days from time of silking	Moisture	Dry matter	Total sugar calculated as invert	Reducing sugar calculated as invert	Non-reducing sugar calculated as invert	Water soluble polysaccharides calculated as dextrin	Total polysaccharides calculated as starch
Golden Bantam:							
5.....	89.45	10.55	4.01	3.36	0.65	0.30	1.43
10.....	88.62	11.38	4.75	3.11	1.64	.28	1.79
15.....	79.14	20.86	5.89	1.92	3.97	3.21	8.44
20.....	69.67	30.33	4.30	.89	3.41	8.90	17.46
25.....	62.98	37.02	2.82	.52	2.30	12.13	23.02
30.....	58.37	41.63	2.14	.67	1.47	13.97	27.73
Howling Mob:							
5.....	89.40	10.60	4.12	3.43	.69	.22	1.21
10.....	88.05	11.95	4.80	3.35	1.45	.18	1.80
15.....	82.29	17.71	5.67	1.90	3.77	2.50	6.47
20.....	71.50	28.50	4.55	.93	3.62	8.96	16.42
25.....	64.80	35.20	3.56	.67	2.89	11.81	21.25
30.....	62.95	37.05	2.06	.61	1.45	12.67	23.88
Dreer's Golden Giant:							
5.....	90.23	9.77	3.91	3.11	.80	.21	1.36
10.....	87.73	12.27	4.50	3.00	1.50	.16	1.78
15.....	79.93	20.07	4.81	1.57	3.24	2.54	8.63
20.....	70.04	29.96	3.88	.84	3.04	8.56	17.43
25.....	65.77	34.23	3.12	.61	2.51	10.56	20.47
30.....	61.73	38.27	2.00	.63	1.37	13.21	26.84
Charlevoix:							
5.....	90.62	9.38	4.05	3.34	.71	.20	1.32
10.....	88.02	11.98	4.61	3.25	1.36	.15	1.64
15.....	82.80	17.20	5.71	1.96	3.75	1.75	5.74
20.....	75.20	24.80	4.16	.76	3.40	7.09	13.41
25.....	69.03	30.97	3.24	.69	2.55	10.31	18.51
30.....	65.84	34.16	2.65	.58	2.07	11.23	21.51
Crosby:							
5.....	90.13	9.87	4.12	3.48	.64	.11	1.30
10.....	88.91	11.09	4.98	3.35	1.63	.14	1.73
15.....	80.61	19.39	6.05	1.93	4.12	2.75	7.30
20.....	70.00	30.00	4.25	.95	3.30	8.86	17.26
25.....	65.20	34.80	3.20	.70	2.50	11.14	20.88
30.....	59.29	40.71	2.20	.53	1.67	14.58	32.48
Morse's Golden Cream:							
5.....	90.19	9.81	3.73	3.15	.58	.16	1.41
10.....	87.79	12.21	4.73	2.57	2.16	.17	1.83
15.....	79.75	20.25	5.43	1.79	3.64	3.26	8.44
20.....	71.99	28.01	4.01	1.04	2.97	8.44	15.67
25.....	64.45	35.55	3.29	.72	2.57	11.48	21.50
30.....	58.52	41.48	2.68	.64	2.04	14.76	29.06
Kelly's Hybrid:							
5.....	90.00	10.00	4.08	3.48	.60	.21	1.39
10.....	88.09	11.91	4.85	2.90	1.95	.17	1.92
15.....	79.73	20.27	5.65	1.38	4.27	3.61	7.77
20.....	70.65	29.35	4.60	.90	3.70	9.05	16.65
25.....	67.46	32.54	3.45	.71	2.74	10.51	19.28
30.....	62.57	37.43	2.80	.52	2.28	13.19	25.55

TABLE III.—Showing results of chemical analyses of 17 varieties of corn sampled at intervals of 5 days throughout the whole growth period—Continued.

Variety and age in days from time of silking	Moisture	Dry matter	Total sugar calculated as invert	Reducing sugar calculated as invert	Nonreducing sugar calculated as invert	Water soluble polysaccharides calculated as dextrin	Total polysaccharides calculated as starch
<b>Hickox's Improved:</b>							
5.....	89.84	10.16	4.15	3.48	0.67	0.53	1.39
10.....	88.39	11.61	4.85	2.88	1.97	.10	1.70
15.....	78.52	21.48	5.73	1.60	4.13	3.25	8.68
20.....	69.37	30.63	3.90	.90	3.00	8.48	16.86
25.....	65.84	34.16	3.00	.64	2.36	11.30	22.34
30.....	62.28	37.72	2.85	.70	2.15	12.80	26.01
<b>Potter's Excelsior:</b>							
5.....	89.42	10.58	4.00	3.42	.58	.31	1.47
10.....	89.05	10.95	4.64	2.90	1.74	.15	1.70
15.....	80.69	19.31	5.23	1.47	3.76	3.23	7.48
20.....	73.13	26.87	4.60	1.07	3.53	7.83	14.78
25.....	66.23	33.77	3.06	.52	2.54	10.13	21.73
30.....	60.99	39.01	2.82	.63	2.19	13.63	26.23
<b>Vaughn's Bantam Evergreen:</b>							
5.....	90.17	9.83	4.13	3.36	.77	.19	1.35
10.....	88.43	11.57	4.72	2.17	2.55	.21	1.82
15.....	82.62	17.38	5.24	1.27	3.97	2.40	6.65
20.....	74.16	25.84	3.95	.92	3.03	7.08	14.34
25.....	68.02	31.98	2.59	.76	1.83	8.42	17.71
30.....	66.13	33.87	2.22	.62	1.60	10.31	21.46
<b>Mammoth Sugar:</b>							
5.....	89.32	10.68	4.26	3.52	.74	.15	1.45
10.....	88.88	11.12	4.78	2.80	1.98	.11	1.91
15.....	81.88	18.12	5.98	1.65	4.33	2.67	6.29
20.....	74.75	25.25	4.24	1.12	3.12	6.91	13.16
25.....	69.86	30.14	3.30	.79	2.51	9.53	17.62
30.....	64.56	35.44	3.04	.67	2.37	11.01	22.49
<b>Old Colony:</b>							
5.....	90.36	9.64	4.00	3.36	.64	.28	1.35
10.....	88.65	11.35	4.23	2.30	1.93	.16	1.64
15.....	83.10	16.90	5.12	1.58	3.54	3.07	6.65
20.....	74.17	25.83	4.24	.96	3.28	7.66	14.10
25.....	71.15	28.85	3.14	.73	2.41	8.55	16.85
30.....	66.52	33.48	2.80	.68	2.12	11.11	21.30
<b>Narrow Grained Evergreen:</b>							
5.....	90.76	9.24	4.01	3.30	.71	.13	1.26
10.....	89.05	10.95	4.58	2.64	1.94	.10	1.59
15.....	83.47	16.53	5.23	2.08	3.15	2.80	6.27
20.....	76.12	23.88	3.97	.97	3.00	6.69	12.92
25.....	72.61	27.39	3.54	.78	2.76	9.27	16.08
30.....	68.72	31.28	2.46	.63	1.83	11.14	19.80
<b>Country Gentleman:</b>							
5.....	90.53	9.47	3.81	3.07	.74	.10	1.38
10.....	88.30	11.70	4.37	2.73	1.64	.09	1.82
15.....	78.60	21.40	5.31	1.49	3.82	3.99	9.12
20.....	71.14	28.86	3.95	1.06	2.89	8.66	16.82
25.....	66.69	33.31	3.02	.75	2.27	11.62	21.76
30.....	61.34	38.66	2.68	.61	2.07	12.86	24.97
<b>Stowell's Evergreen:</b>							
5.....	91.01	8.99	3.82	3.14	.68	.10	1.24
10.....	89.26	10.74	4.60	2.64	1.96	.12	1.65
15.....	82.24	17.76	5.68	1.38	4.30	3.04	6.77
20.....	75.97	24.03	4.09	.96	3.13	7.56	12.90
25.....	72.07	27.93	3.38	.72	2.66	9.19	16.48
30.....	66.82	33.18	2.23	.70	1.53	12.66	21.95
<b>Reid's Yellow Dent:</b>							
5.....	90.17	9.83	3.62	3.00	.62	.05	1.55
10.....	89.73	10.27	3.95	2.84	1.11	.31	1.67
15.....	86.75	13.25	4.47	2.06	2.41	.25	4.85
20.....	75.75	24.25	2.90	1.10	1.80	.68	13.64
25.....	67.95	32.05	2.33	.64	1.69	.81	21.49
30.....	62.44	37.56	2.07	.43	1.64	1.04	27.90
<b>Boone County White:</b>							
5.....	89.85	10.15	3.73	3.27	.46	.08	1.28
10.....	89.60	10.40	4.28	3.45	.83	.10	1.62
15.....	86.44	13.56	5.84	2.68	3.16	.12	3.74
20.....	80.83	19.17	4.02	1.41	2.61	.34	9.34
25.....	73.45	26.55	3.52	1.18	2.34	.57	16.07
30.....	65.63	34.37	2.79	.83	1.96	.72	22.54

## DISCUSSION OF ANALYTICAL DATA

## MOISTURE CONTENT

It will be seen from the tables that in all varieties the moisture content decreases throughout the growing and ripening period of the ear. In the early stages the moisture content is fairly uniform for all varieties, but later on considerable differences are observed. These differences seem to be more or less closely correlated with the rate of maturing of the varieties. Golden Bantam, Dreer's Golden Giant, and Crosby are among those maturing most rapidly, and their moisture content is low during the later stages. Stowell's Evergreen, Mammoth Sugar and Old Colony are among the slow-maturing varieties and their moisture content during the later stages is seen to be somewhat higher.

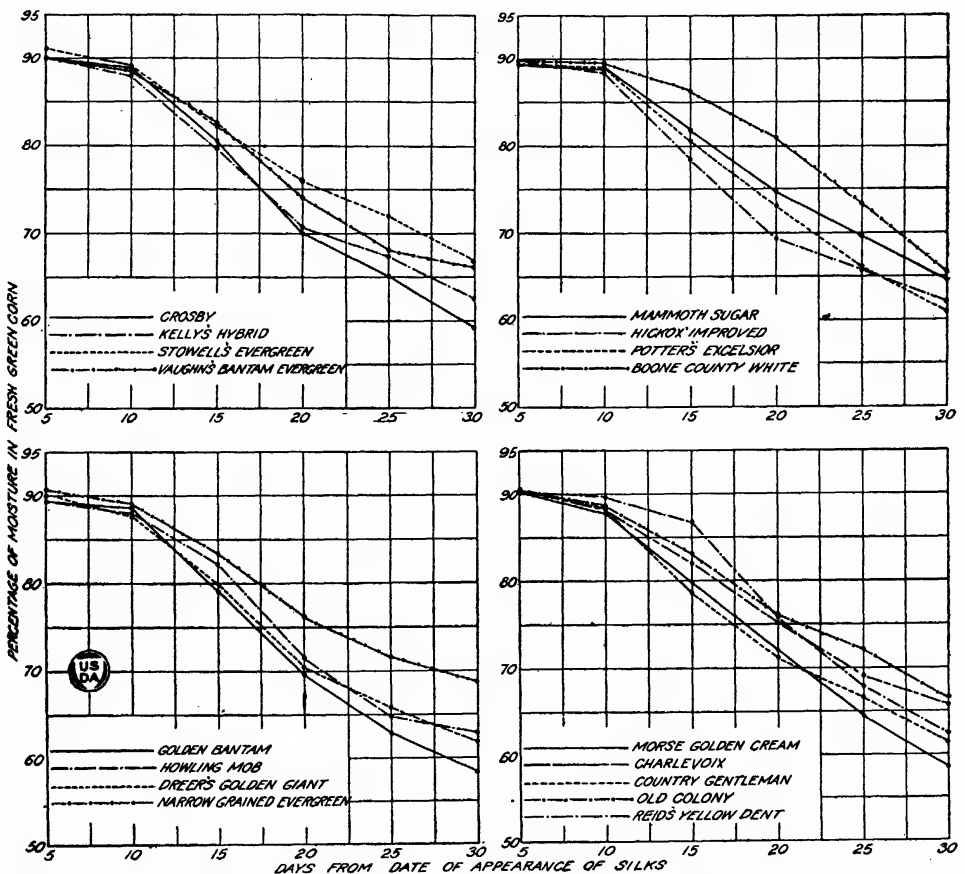


FIG. 9.—Curves showing the variations and changes in moisture content of the kernels of 15 varieties of sweet corn and 2 varieties of field corn during the development of the ears

Other varieties which are intermediate in rate of maturing are also more or less intermediate with respect to their moisture content. The two dent corns were even slower in development than Stowell's Evergreen and had correspondingly high percentages of moisture. It should be stated here, however, that the differences in the rate of development of the ear among the sweet corn varieties were much less than anticipated, and even at the 20-day stage are seen to be comparatively small. The principal difference between early and late-maturing corns lies in the length of time the plants require to come into flower rather than in the rate of development of their respective ears. Variations in the moisture content of the different varieties of corn at the various stages of their development are shown in the curves of figure 9.

## SUGAR CONTENT

The total sugar is found to increase up to the 15-day stage and then to decrease rapidly at first and then more slowly during the later-maturing stages. The proportion of the reducing to nonreducing sugars is constantly changing. The reducing sugars decrease throughout the growing and maturing periods of the kernels. The nonreducing or cane sugar is low at first, increases rapidly to 15 days and slowly decreases. An idea of the rate and nature of these changes may be obtained at a glance from figures 10 and 11.

The different varieties of sweet corn do not vary widely at any stage of maturity in the quantities of sugar which they contain. At the ages of 5 and 10 days differences in amount are slight. The greatest variation seems to be at 15 days. With but a few exceptions the differences in sugar content at 20 days can not be considered as very significant. Kelly's Hybrid, Potter's Excelsior, and Howling Mob had the highest sugar content at this stage and Dreer's Golden Giant, Hickox's Improved, and Country Gentleman the lowest. In the slow-maturing varieties the sugar content decreased almost as fast as in the more rapidly maturing corns. This was contrary to expectation, and if found to be true from year to year, must be taken into account in choosing the proper stage for canning. Of the field varieties Reid's Yellow Dent is seen to be significantly lower in total sugars than the sweet varieties, but the differences between these and the Boone County White is not very marked. There is, however, at 20 and 25 days a lower percentage of nonreducing or cane sugar in both of the field varieties.

The relation of these analytical results to the findings in the quality of the canned samples will be discussed later in the consideration of that subject.

## POLYSACCHARIDES

The nature and quantity of the polysaccharides in sweet corn, together with the moisture content, determine to a large extent the consistency of the canned product. They are, therefore, of considerable interest and importance in canning. In food value the three most important groups of the polysaccharides are the starches, dextrins and pentosans. The last-mentioned group is reported to be present in but small amounts in the maize kernel (5, 60, 63, 69) and from the standpoint of canned corn do not seem to be significant. The present study has been confined, therefore, to the starches and dextrins.

From the analytical tables it is noted that in all varieties the content of total polysaccharides increases with age of the corn, but the rate of increase varies somewhat with different varieties. The slow-maturing corns, such as Stowell's Evergreen, Narrow Grained Evergreen, Mammoth Sugar, and Old Colony show a less rapid increase in the total polysaccharides than is observed in Golden Bantam, Dreer's Golden Giant, Crosby and others of the more rapidly maturing sorts. This is closely correlated with the differences in moisture content and, as will be shown in connection with the discussion of the canned material, with the consistency of the canned product.

Of the two field varieties the total polysaccharides in the Boone County White is somewhat below the average of the sweet varieties and in Reid's Yellow Dent it is no greater than in many of the sweet varieties. Great differences are observed, however, in the consistency of the canned product from the sweet and the field varieties, and since the total polysaccharides show no marked differences in quantity the nature of the polysaccharides must be important. It would seem that the amount of the water soluble portion might throw some light upon this matter. In the sweet varieties at 20, 25, and 30 days of age the water soluble portion varies from less than one-half to a little more than one-half of the total

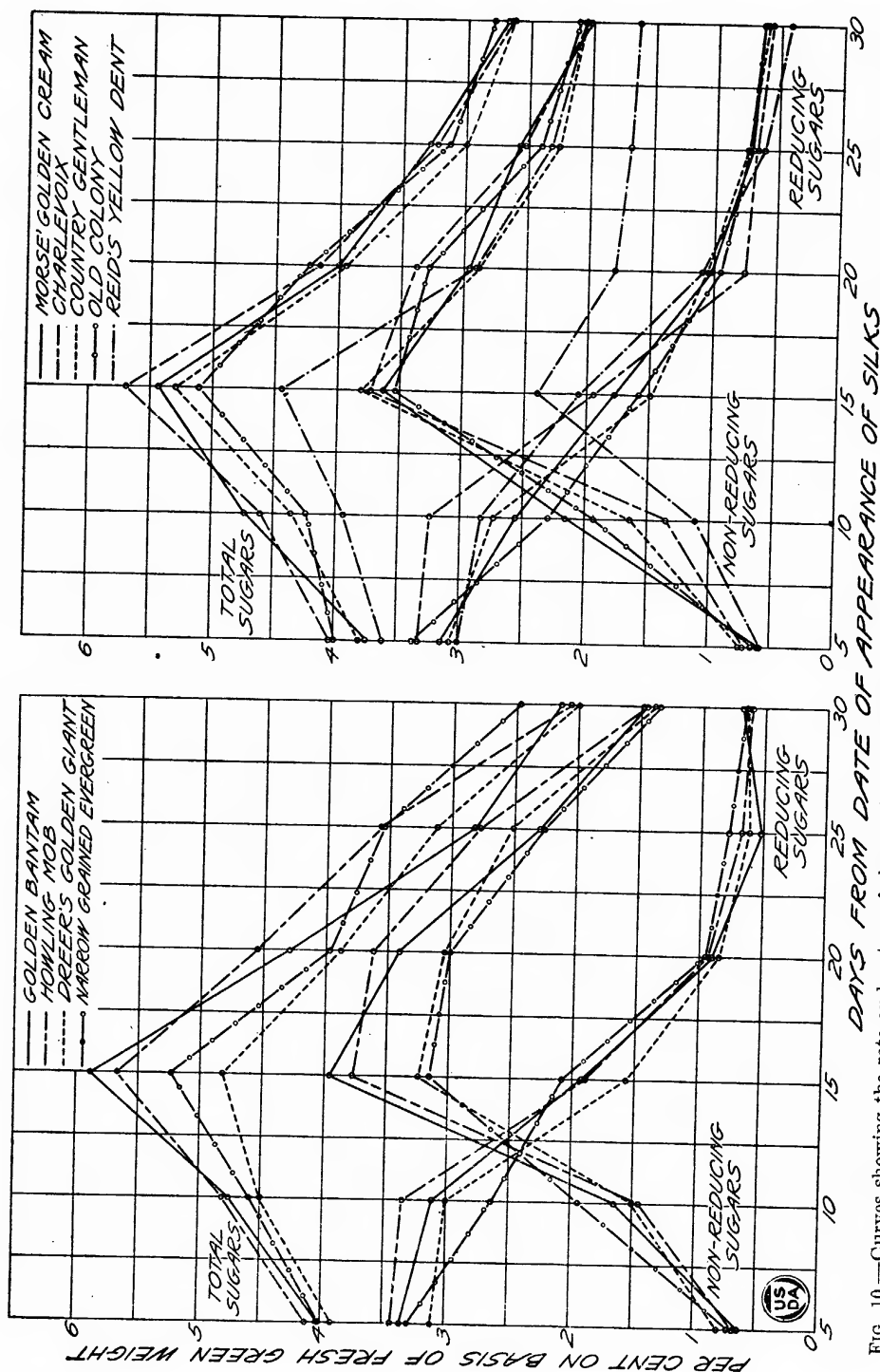


FIG. 10.—Curves showing the rate and nature of changes in sugar content of Golden Bantam, Howling Mob, Dreer's Golden Giant, Narrow Grained Evergreen, Morse's Golden Cream, Charlevoix, Country Gentleman, and Old Colony sweet corn and Reid's Yellow Dent field corn during the development of the ears.

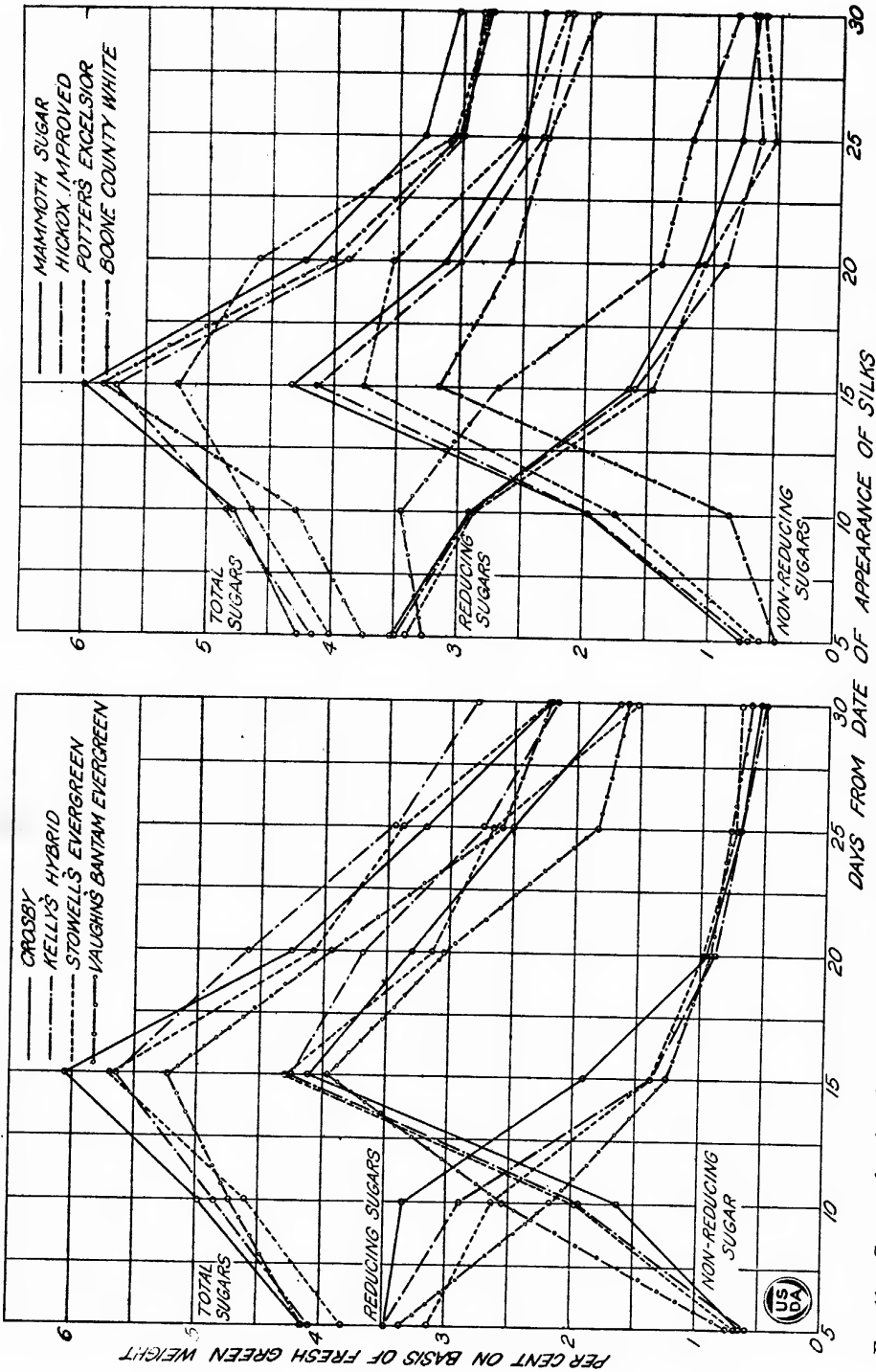


Fig. 11.—Curves showing the rate and nature of changes in sugar content of Crosby, Kelly's Hybrid, Stowell's Evergreen, Vaughn's Bantam Evergreen, Mammoth Sugar, Hickox's Improved, and Potters Excelsior sweet corns and Boone County White field corn during the development of the ears



polysaccharides, while the amounts present in the field varieties are very small, usually less than 1 per cent. The water soluble portion seemed so important that it was thought worth while to learn something more about its chemical nature.

Dextrin was reported by Salisbury (54) as occurring in a number of varieties of maize. He found that nearly half of the total polysaccharides of sweet corn was dextrin, while the field corn showed but a small amount; and he attributed the wrinkled condition of mature corn to the shrinkage of the kernels through the loss of water from the solution of dextrans and albumins which they contained. The proportion of starch to the dextrin was found to be greater in the early stage of development which he considered as evidence that starch is formed first and is later changed to dextrin. Correns (20) also reported the presence of dextrin in sweet corn and pointed out its significance in maize crosses. Atwater (5) did not determine definitely whether dextrin was present in sweet corn but came to the conclusion that it was absent. Harper (28) stated that the wrinkled character of sweet corn was due to the larger proportion of sugar, dextrin, and gums present. Weatherwax (71) reported the presence of dextrin in waxy maize but was not able to determine definitely whether it was present in sweet corn.

A complete chemical examination of the cold water soluble portion of the polysaccharides was not attempted in this work, but a number of experiments were performed which yielded significant results. The residue after extraction with 70 to 80 per cent alcohol dissolves partially in cold water giving an opalescent or milky white solution which is very difficult to filter. If allowed to stand for several hours and the supernatant liquid then filtered, there is obtained a faint to distinct blue color when a drop of very dilute iodine solution is added. If a drop of 1 per cent solution of iodine is added a characteristic red is usually instantly obtained. Results vary somewhat with the different corns and their degree of maturity. It is apparent that both dextrin and soluble starch are present.

An attempt was made to separate the dextrin and the soluble starch quantitatively by precipitating the soluble starch with basic lead acetate, determining the dextrin directly and obtaining the figure for soluble starch by subtracting this from the total water soluble polysaccharides, but when basic lead acetate was added to the sample it became more turbid and the small amounts of precipitate settled out very slowly and filtration was difficult. Owing to this, clear-cut separations could not be made and duplicate determinations did not check closely. However, the figures obtained in this way for the different varieties showed 1 to 3 per cent less dextrin than the total amount of water soluble material. It seems apparent from this and other qualitative tests that the varieties differ considerably in the proportion of dextrin to soluble starch. By allowing the solutions to stand for several weeks after the addition of the basic lead acetate, filtering the supernatant portion, and removing the lead, a product was obtained which gave only the red color characteristic of dextrin. This substance was not precipitated by half saturation with ammonium sulphate, was precipitated by alcohol, had adhesive properties, and on hydrolysis yielded reducing sugars.

In the 5 and 10 day samples nearly all the water soluble portion was precipitated by basic lead acetate, and since the aqueous extract rarely gave a blue color with iodine it was concluded that the water soluble portion in the very early stages of development of the kernel was made up principally of pentosans or gums.

In the two field corns the water soluble portion gave a blue color with iodine, and was precipitated by basic lead acetate, and also by half saturation with ammonium sulphate thus indicating soluble starch; and in the later stage of maturity the water soluble polysaccharides in the field varieties appeared to consist entirely of starch.

Distinct and significant differences exist, therefore, between the polysaccharides of the sweet and the field varieties of corn, which differences are believed to be of very great importance from the standpoint of table quality.

#### TESTS UPON CANNING QUALITY

The sampling of the different varieties of corn for canning tests was begun upon ears at 15 days from the date when their silks first appeared and subsequent samples were taken at age intervals of 5 days, up to and including 30 days. The ears were plucked in the morning and taken at once to the laboratory where they were husked, silked, trimmed when necessary, and washed. The corn was cut from the cob "Maine style," that is, the outer one-third to one-half of the kernels cut off and the remaining edible portion scraped from the cob. In order that careful study might be made of the relation of the degree of maturity to the consistency of the canned product it was necessary that each can receive the same amounts of liquor and corn. Therefore, instead of mixing the cut corn and liquor in a precooker each was heated separately and measured quantities placed in each can. No. 2 "sanitary top" cans were used. The corn was pre-cooked in steam heated vessels with frequent stirring until the temperature reached 85° to 90° C. (185° to 194° F.) and the liquor was heated in glass flasks to the same temperature; 120 cc. (4.2 ounces avoirdupois) of the hot liquor<sup>7</sup> was first placed in the can and 480 gm. (16.9 ounces avoirdupois) of the corn added, thus giving a 1:4 mixture. By putting the hot liquor into the can first a better mixture with the corn was secured than when the corn was introduced before the liquor. The cans were sealed immediately and processed as rapidly as the capacity of the equipment permitted. Those cans which could not be processed at once were held in a water bath at about 80° C. (176° F.) until they could be placed in the retort. The cook or process was for one hour at 121° C. (249.8° F.) in the steam retort, at the end of which time the cans were removed from the retort and submerged in cold water to cool.

At the end of the canning season and at irregular intervals thereafter series of cans representing all the varieties studied at their various stages of maturity were opened and subjected to critical comparative study by various groups of individuals qualified by experience and training to judge of the quality of the canned product. The results of these tests were as follows:

At 15 days maturity all varieties of sweet corn yielded a tender and sweet product but it was lacking in body and flavor and was classed as slightly too immature to grade as first-quality canned corn. Golden Bantam, Dreer's Golden Giant, and Morse's Golden Cream of the yellow and Crosby and Hickox's Improved of the white varieties were not far, however, from being in prime canning condition at 15 days.

At the 20-day stage all the varieties were judged to be in about prime canning condition, both from the standpoint of body or consistency and of flavor. Slight differences in sweetness were observed among the varieties and the consistency of the product varied somewhat, but aside from the differences in size of the kernels among some of the corns which made it possible to identify them readily, variations in flavor, sweetness, and with few exceptions, consistency, were too small to be of practical significance.

Among the later maturing varieties such as Stowell's Evergreen, Narrow Grained Evergreen, Mammoth Sugar and Old Colony the consistency of the canned product was a little thinner than with the earlier sorts. The explanation for this is to be found in the greater moisture content of these varieties as shown by the figures in Table III. The flavor and sweetness of both the earlier and the later corns were remarkably uniform and were found to be largely independent of the consistency of the canned corn.

<sup>7</sup> The liquor was prepared by dissolving 2½ per cent of salt and 6¼ per cent of sugar in distilled water.

At the 25-day stage all varieties were past the prime canning condition from the standpoint of flavor and sweetness, and, with the exception of some of the later maturing varieties already mentioned, from the standpoint of consistency also. The kernels were tougher and the corns had lost much of their desirable flavor.

Of particular interest is the matter of the consistency of the product from the slow maturing varieties. At 25 days the consistency of their product was about the same as that of the earlier maturing corns at the 20-day stage. It is a commonly accepted idea that Stowell's Evergreen and some other varieties more or less closely related to it remain in canning and table condition for a longer time than do some of the earlier sorts, and from the standpoint of consistency of the canned product this has been found to be true. It should be pointed out however, that the sweetness and flavor are not maintained to the same degree, for, as may be seen by the table on the chemical composition mentioned above, the sugar content falls almost as rapidly in these varieties as in the others, and at the same age of the ears. Therefore, corn which may appear to be still in good table condition may yield a canned product of poor quality.

At 30 days all the varieties were far past the canning stage and further comment upon them is unnecessary.

One other significant fact which deserves mention was brought out in these comparative canning tests. At the outset of these investigations it had been expected to find that the product of such varieties as Golden Bantam and Crosby would be sufficiently distinct in flavor and other qualities from the other varieties as to make them characteristic and distinct, but this was not found to be the case with the corns used in the present comparative study. Whether this would hold true for the same varieties grown in other sections of the country is not known, but it is believed, however, that the degree of maturity rather than the variety used will be found to determine the quality of the product irrespective of locality where grown.

The results of canning tests upon the two dent corns are of considerable interest. These corns were slower in coming to the full milk stage than the sweet varieties, but Reid's Yellow Dent was considerably earlier than Boone County White. From the standpoint of the appearance of the contents of the raw kernel Reid's Yellow Dent would have been considered in prime canning condition at between the 20- and the 25-day stages. Boone County White, however, was still in the milk stage at 30 days.

It will be seen by reference to Table III that Boone County White at the 20-day stage has as high a total sugar content as many of the sweet varieties and by reference to the puncture tests (fig. 8) that the kernels were not particularly tough at this stage. A reasonably acceptable product therefore might have been expected. The canned product from this variety at 20 days was found to be sweet and of good consistency, but the kernels were distinctly harder than was the case with the true sweet varieties. The flavor of the product was somewhat inferior to that of the sweet corns, but the canned corn was nevertheless edible. At the 25-day stage the corn was nearly solid in the can and the kernels were hard and tough. The flavor was not unlike that of hominy. At the 30-day stage the corn was very hard and horny in texture, and it was only with difficulty that a spoon could be introduced into the canned product of both the 25- and the 30-day stages. It is of particular interest to note that at even 30-day stages the raw corn was still in the milk stage and by the thumbnail test would have been considered in good condition for canning.

The product from Reid's Yellow Dent was inferior to that from the Boone County White at all stages, being less sweet, poorer in flavor, and unattractive

in appearance. This variety at 25 days was still in the milk stage, but the product from the corn was hard and unpalatable even at 20 days.

From these results it seems apparent that some factor or factors other than moisture content and toughness of the kernel in the raw state determine the consistency and texture of the product from these varieties of field corn. Whether this would hold true for all field varieties remains to be determined.

### GENERAL DISCUSSION

For a proper evaluation of the foregoing data certain general considerations must be held in mind, and before final conclusions are drawn from the work what appears to be significant facts deserve more attention than they have thus far received in the present treatment of the subject.

### ENVIRONMENTAL CONSIDERATIONS

At the outset it should be remembered that with the exception of the comparative canning tests of preceding seasons the bulk of the work upon which the present report is based was confined to one season only, and the findings are known to apply, therefore, to but one location and one set of environmental conditions. While it is believed that the main facts brought forward here would be found applicable to all parts of the country, it is fully realized that a similar line of experimentation conducted in some other section of the country, or even in the same place but with differing conditions, might and doubtless would yield somewhat different results from those obtained in the present case. For example, temperature is known to have a very great influence upon the development of the maize plant, and without doubt it also materially affects the rate at which the corn matures in the ear. For this reason it is probable that in a more northern latitude the rate of coming to canning maturity would be somewhat slower than was found in the present instance. The amount and distribution of rainfall is likewise known to exert a powerful influence upon the progress of corn development, a severe drought during the latter part of the growing season materially hastening the ripening processes.

In view of the very important relation between maturity of the corn and the quality of the canned product as brought forth in this report, it seems highly desirable that similar work be done in the various sweet-corn-producing sections of the country to the end that canners may more effectively control the quality of their product by the consistent handling of the raw corn at the proper stage of maturity. The bringing together of such information from various sections of the country would likewise be of great scientific value by throwing light upon the physiology of the developing corn plant.

Another fact of importance to remember in the present connection is that corn as a plant is not only very responsive to environmental conditions but also that in the hands of growers and breeders the characteristics of a particular variety may be very materially altered in a few seasons. Many different strains of certain varieties have arisen in this way which are sufficiently unlike in their behavior to make it impossible to judge of the qualities of all by the observation of one. It has very frequently been claimed that corns from the same strain produced in different parts of the country behave differently when grown side by side. Therefore, it is probable that other strains of the varieties studied in the present case, or even the same strains the seeds of which had been secured from different sources, would have yielded different comparative results from those obtained in the present instance, even though grown under identical conditions. This emphasizes again the need for more experimental work along these lines on the spot where the various strains have been adapted to local environments.

## WHEN IS CORN AT THE BEST CANNING STAGE?

To determine at what stage of maturity corns were in best condition for canning studies were made along several lines. The condition of the corn as indicated by physical development received careful attention in order to discover at what stage the highest yields in cut corn would be obtained and at what stage it would seem most advantageous to harvest the crop; chemical analysis of the cut corn was made at all stages of development that it might be learned at what period the amount and the constitution of the carbohydrates would indicate highest quality; and finally sample packs of the different varieties at various stages were prepared so that a test of the comparative table qualities of the canned products might be made. The close correlation of the findings from these three lines of study is of considerable interest.

From the standpoint of safe practice it would seem that the most favorable time to harvest corn for canning purposes would be at or very shortly after the time when the rate of development of the kernels had reached its peak, as beyond this time the rate of increase falls off rapidly and the hazards of successful packing are increasingly great. Careful study of the figures giving the percentage of cut corn from the husked ears at the different stages (see Table I) obtained in these studies, shows that in the great majority of cases the peak of the rate of development occurred in this instance at between 15- and 20-day stages, in no case occurring beyond the 25-day stage. This result is in close agreement with those supplied by the chemical analyses.

It is common knowledge that high quality in canned sweet corn is closely associated with high natural sugar content, and that added sugar does not give the same pleasing result as the sweetness supplied by nature. It is apparent therefore, that corn should be canned while the sugar content is high, and if possible, at the time when it is present in greatest amount. The chemical analyses made of the different varieties of sweet corn at age intervals of 5 days, in the present studies, showed that the highest point in the development of sugar under the present conditions was reached at the 15-day stage. This was found true for all varieties regardless of the time of planting or the earliness or lateness of the corn. It seems, therefore, that from the standpoint of natural sweetness canning should not be delayed for any considerable period beyond the 15-day stage.

These two lines of evidence are supported by a third, namely, the canning tests which have just been discussed. These all combine to point to a rather definite, brief period represented by the 20-day stage, or a few days earlier for rapid-maturing strains, during which it is possible to attain the highest quality in the canning of corn. Beyond this point the increase in yields are small, the products are lacking in natural sweetness, the desirable flavor is impaired and the kernels become increasingly tough.

## THE RELATION OF SUGAR CONTENT TO SWEETNESS

In the consideration of the relation of the sugar content as indicated by chemical analysis, and the sweetness as indicated by taste, it needs to be pointed out that the total sugar content of a particular substance is not an exact measure of its real sweetness. Two factors are operative here, namely, the kind of sugar present and its concentration. Since cane sugar has higher sweetening power than dextrose, or invert sugar, changes in the relative proportions of these affect the sweetness even when the figures for total sugars present are the same. Since, also, the moisture content is constantly changing in the corn the percentage of sugar as shown by the analyses is not a real measure of the concentration. The per cent of sugar at the 15-day stage was found to be considerably higher than at 20 days,

but the difference in sweetness was much less marked than the figures would indicate. In the Boone County White at the 15-day stage the percentage of sugar was found to be about equal to any of the sweet varieties at this stage, but it was distinctly less sweet to the taste, due, apparently, to its higher moisture content, and hence a greater dilution of the sugar and to its smaller proportion of cane sugar. Where moisture content and other constituents are about equal, differences in sweetness are more nearly proportional to the differences in the percentages of sugar. In such cases the higher the percentage of sugar the higher the quality of the product.

There are, probably, other factors which affect the relation of sweetness to the percentage of sugar as practically all the 30-day samples of canned corn appeared less sweet to the taste than would have been indicated by the sugar content.

#### THE THUMBNAIL TEST

Judging of the chemical composition of corn in the field by the appearance of the ear or by the application of the common thumbnail test leaves much to be desired. When the test is applied to a number of varieties of the early, medium and late sorts it is not dependable and may be entirely misleading. It may be and is applied with considerable accuracy in judging of the maturity of a single strain with which the person making the test is familiar, but so great have been found the differences in composition of different varieties giving comparable results with the thumbnail test that except for indicating what would be the consistency of the canned product from the sweet varieties it proved of but little value. For instance, Crosby corn at the 20-day stage by the thumbnail test would have been judged to be at about the same stage of maturity as Stowell's Evergreen at the 25-day stage, but the chemical analyses showed that they were far from having the same composition particularly with respect to sweetness.

When used upon field varieties the test was found still more unreliable as it did not prove a safe index even of what the consistency of the canned product would be. Boone County White field corn at the 30-day stage would have been judged by this test to be only slightly more mature than Crosby at 20 days, and yet the consistency and texture of the products from the two varieties at these stages were as unlike as could be imagined; the Crosby being excellent in body and texture and the Boone County White so solid and tough that it could be removed from the can with a spoon only with great difficulty.

Failure of workers to take these facts into consideration has made the proper interpretation of their results extremely difficult if not impossible. Canning maturity is more closely associated with the age of the ear than with the physical condition of the kernel contents. Therefore, it is particularly important in experimental work that the investigator know as closely as possible the exact age of his material.

#### IMPROVEMENT OF VARIETIES

One of the chief values of a study of this sort is the help it affords by indicating the line of procedure which should be followed in the improvement of varieties of corn used for canning purposes.

In view of the importance of the tenderness of the kernel to the quality of the canned product it is believed that no greater service could be rendered by sweet-corn breeders than by giving attention to the development of tender strains from the standard canning varieties.

From the figures upon percentage yields of cut corn it is of interest to note that for both the yellow and the white varieties the highest yields were produced in this instance by the close-grained or many-rowed corns having deep kernels and correspondingly small cobs. The closer the kernels are set in the ear the

greater the yield is likely to be, providing, of course, the grains are of good depth. Increase in the number of rows to the ear and great depth of grain, together with greater tenderness are things to be particularly sought in sweet corn selection and breeding.

From the standpoint of sweetness and flavor in the canned product the corns producing quickly maturing ears seem to have the advantage over the slowly maturing sorts, the corn coming to canning maturity more closely to the time when the sugar content is greatest. Breeding for this character would, therefore, seem desirable.

Owing to the resistance of some of the late corns to bacterial disease there seems to be a need, also, for the selection of strains which will maintain a higher sugar content at the 20 and 25 day stages, thus making possible the utilization of these corns at later stages when greater yields of first-quality corn might be obtained. In this connection it seems, also, that the development of strains having a higher water-soluble polysaccharide content would be worth striving for. The crossing of the sweet with the waxy varieties having a very high dextrin content would seem to offer possibilities in this line.

Attention has already been directed to the rate at which different varieties of sweet corn under study came into flower, and it was noted that while silking in all the varieties continued for two weeks or more, in some the bulk of the silking occurred within a very few days, as, for instance, Golden Bantam and Country Gentleman. Although, as already mentioned, environmental factors, quality of the seed, etc., may have had some influence upon this particular result, it has seemed that a varietal characteristic was exhibited here which is worthy the attention of the corn breeder.

For home and market garden purposes an irregular or prolonged flowering period may be of advantage, as it increases correspondingly the number of days when corn may be picked in prime condition for table use. The reverse is true for corn grown for factory purposes. Several pickings from the same field is hardly feasible for the average farmer, and therefore the corn ordinarily delivered at the factory contains a considerable percentage of immature and overmature ears, which affect to a greater or less extent the quality of the product derived from it. Therefore, from the standpoint of large yields, high quality of the canned corn, seasonal hazards, etc., it is desirable that all the plants flower at as nearly the same time as possible, and that the subsequent development of the ears be highly uniform. It is believed that this characteristic of prompt development of the ears may be readily attained by careful selection in the existing varieties.

#### PREMIUM CORN

In conclusion, one other matter calls for serious consideration. It must be realized that all attempts in the attainment of high quality in canned corn are dependent upon the degree of cooperation between the grower of the corn on the one hand, and the canner on the other. The farmer grows the corn for the financial return it brings and it is natural that he should desire to realize as much as possible from the crop. Most farmers know that where compensation is based purely upon the weight of cut-off corn it is to their advantage to delay the harvesting as long as possible. Such a condition of affairs is inimical to the production of a first-quality canned product. From the standpoint of the canner, corn in prime condition is worth more than either immature or overmature corn and he must secure it in this condition if his pack is to be of the highest quality. Therefore, it would seem only fair to both grower and canner that quality be made the basis of compensation, the highest price being paid for corn in that stage of maturity which will yield a canned product of the finest quality.

## SUMMARY

1. (a) In the maize plant the flowering period appears to be the most significant time from which to reckon its rate of development and maturity.

(b) In any variety, even when planted upon the same date, individual plants vary as much as two weeks or more in the length of time necessary for them to reach the flowering period. Thus, when a field of corn begins to come into flower the number of silks that appear each day increases for 6 or 7 days when a maximum is reached and then falls off until the end of the flowering period. Part of this appears to be due to environmental conditions and part to inherent variations.

(c) Up to 20 or 25 days of age the development of the individual ears silking at the same time appears to vary much less than the time of flowering, though small differences in this respect have been observed.

(d) The length of time between planting and flowering varies with the date of planting and depends upon temperature, rainfall, and other factors. Likewise, the rate of development of the ear is influenced to some extent by differences in seasons.

(e) There is often a wide difference among the varieties in the length of time between planting and flowering which largely accounts for their earliness or lateness. In the present studies among those varieties flowering early were Golden Bantam, Dreer's Golden Giant, Crosby, and Howling Mob, while Country Gentleman, Stowell's Evergreen, and Narrow Grained Evergreen flowered late.

(f) There is a small but often a distinct difference in the rate of developing and maturing of the ear in varieties silking at the same time. In these experiments Stowell's Evergreen and Country Gentleman flowered at approximately the same time, but the rate of development of the ear was somewhat different, the Country Gentleman maturing more rapidly.

(g) In the study of sweet corn varieties and their development it has been found advantageous to take samples for analysis and for canning from selected ears of the same age as determined by the date of silking.

2. (a) Data have been obtained upon 15 varieties of sweet corn and two varieties of field corn with respect to the weight of the ears and the proportions of cut-off corn and cob to both the husked and the unhusked ears at intervals of 5, 10, 15, 20, 25, and 30 days from the date of silking. Since many factors may cause variations from year to year in results of this sort and a relatively small number of ears were used in the tests, too close analysis of the figures must not be made. Great care was exercised in selecting the ears so that the figures would be as accurate as possible and it is believed that they indicate the general progress of changes in ears of corn under most conditions.

(b) The weight of the ear, and what is even more important the weight of the cut-off corn, continues to increase even 30 days after the date of silking. The most rapid increase was found to take place at between 15 and 20 days in the rapidly maturing corns and at between 20 and 25 days in the slow maturing varieties.

(c) Country Gentleman gave the highest proportion of cut-off corn and Golden Bantam the lowest in these tests. The small number of rows to the ears in this latter together with the greater proportion of husk probably accounts for this result. Other varieties giving high proportions of cut-off corn were, Stowell's Evergreen, Morse's Golden Cream, Narrow Grained Evergreen and Old Colony. Of the early varieties Crosby, Dreer's Golden Giant and Charlevoix gave fair yields. Few rows to the ear and shallow kernels result in low yields.

(d) No data were obtained upon the total yield per acre. The figures given in these records are based upon average ear yields which may or may not have any relation to total yields of varieties per acre.



3. (a) Information upon the comparative toughness of the kernels of different varieties of corn was obtained at 5-day age intervals throughout the growing and maturing period by means of an instrument devised specifically for the purpose. This measured the toughness of the kernels by showing the amount of their resistance to puncture. The features of this instrument are described and illustrated in the text.

(b) In general there is a relation between the resistance to puncture of the fresh corn and the toughness of the canned product made from it. Since the resistance to puncture increases with age, tests of this kind may be used to indicate in a general way the age of the corn which may facilitate the selection of the proper stage for canning.

(c) The variations in toughness among different corns of the same age were found to be relatively small, though in a few instances, as with Crosby, a distinct varietal difference in this character was observed.

(d) Factors other than toughness of the pericarp may affect the texture of the grain in the canned product as was shown in the case of the product from the two field varieties in which the kernels were found to be considerably harder than the puncture tests would lead one to expect.

4. (a) In all the varieties of sweet and field corn tested the moisture content of the kernels decreased continually during the growth and maturing periods. The rate of decrease varied considerably in different varieties, the rate being most rapid in Golden Bantam, Dreer's Golden Giant and Country Gentleman, and slowest in the Evergreens, Mammoth Sugar and Old Colony. Of the field varieties Boone County White showed a much slower decrease in moisture than Reid's Yellow Dent. The relative rates in the decreases in moisture in the different varieties was found to correlate closely with the relative rates in maturing as shown by field observations and the character of the product canned at various stages. The moisture content is also closely correlated with the consistency of the canned product of the sweet varieties, both as regards different varieties and different stages of maturity.

5. (a) The percentage of sugar in the kernels of green corn was found to change constantly during the development and ripening of the ear. The total sugar continued to increase up to 15 days from appearance of silks and then decreased rapidly throughout the maturing period. The reducing sugars were high at first and steadily decreased throughout the growth and maturing periods. In contrast to this the cane sugar increased rapidly at first to the 15-day stage and then slowly decreased as the corn approached maturity. The general order of these changes was practically the same for all varieties, the slowly maturing varieties decreasing in sugar content nearly as rapidly as those maturing more quickly.

(b) These changes in the percentage of sugars profoundly affect the sweetness of the canned product, though the percentage of total sugar is not an exact measure of the sweetness. This is determined rather by the nature and concentration of the sugar. That the composition of the sugar itself is changing is manifest by the change in the ratio of reducing to nonreducing sugar. Also the moisture content is constantly changing in the corn so that the percentage of sugar is changed without necessarily changing the concentration. These influences are manifested in the quality of the products canned at the 15 and 20 day stages. The percentage of total sugar in the corn at the 15-day stage is much higher than at the 20-day stage but the difference in the sweetness of the two products is not as great as the percentage of sugar would indicate.

6. (a) The total polysaccharides were found to increase continuously throughout the development and ripening of the ear of corn. As the nature and concentration of sugars were found to affect the sweetness of the canned product, in like manner the amount and nature of the different polysaccharides were found

to affect profoundly the consistency of the canned product. The sweet varieties contain a high percentage of water-soluble polysaccharides which appear to consist of dextrin-like substances mixed with varying amounts of material similar to soluble starch. In field corns these water-soluble polysaccharides were present in but small quantities and that principally in the form of soluble starch. When canned at comparable stages of maturity the kernels of the field corn were harder and tougher than any of the sweet varieties. This striking difference in cooking quality seems to be due partly to the difference in the nature of the polysaccharides.

7. (a) The so-called "thumb-nail test" proved to be unsatisfactory in the selection of corn for canning. It did indicate the rate of maturity and the moisture content, but it was not a safe guide in estimating the sugar content, especially when applied to different varieties.

(b) If the "thumb-nail test" were used to determine the best stage for canning, Golden Bantam and other rapidly maturing varieties would probably yield a better canned product than Stowell's Evergreen and others of the slowly maturing sort.

8. (a) Observations of three seasons lead to the conclusion that Stowell's Evergreen and allied strains do remain green and succulent for a longer time than Golden Bantam and some of the earlier corns. The "evergreen" property, however, may lead to the canning of an inferior product, as there is a tendency to delay the canning of these varieties longer than the early sorts. From the analytical results of one season it seems that the sugar content decreases almost as rapidly in the slow maturing as in the rapidly maturing varieties. This must be taken into account when considering the merits of the slow-maturing types.

9. (a) The different varieties of sweet and field corn were also subjected to comparative canning tests to determine their relative merits for canning purposes and to find at what stage of maturity they were in best canning condition. Canned samples were prepared at intervals of 15, 20, 25, and 30 days from the date of silking, and complete series of samples from these tests subjected to the critical examination of judges qualified by training and experience to pass upon their relative merits. Their findings are embodied in the conclusions here presented:

The 15-day samples were very sweet and tender; but were thin, watery, and lacking in flavor. However, the rapidly maturing varieties, such as Golden Bantam and Crosby, were only slightly too immature to be graded highest in quality.

The 20-day stage samples were still quite tender and sweet and of good flavor, but little variation was noted among the varieties in this respect. All exhibited a more desirable consistency than at 15 days, but Golden Bantam, Crosby, and others that mature rapidly gave a product of a little heavier consistency than did the slow-maturing types, such as Stowell's Evergreen.

At the 25-day stage the corns were tougher, less sweet, and the flavor less pleasing than at the 20-day stage. The rapidly maturing varieties yielded products of too heavy a consistency to grade as first quality, but the slowly maturing types were thinner and about the same as of the early maturing varieties at the 20-day stage.

At the 30-day stage all varieties were too mature for canning, the product in all cases being dry, tough, and very poor in flavor.

In spite of the above-mentioned differences in varieties they were very nearly the same in their general quality. Differences were found to be too slight to be significant, it being found practically impossible to identify varieties by quality alone. When canned at the same age, especially at 20 days, it was very difficult to decide which variety should be given first place, and judges differed widely in their choice.

(b) There was unanimous agreement that corns at the 20-day stage yielded a better canned product than at any other stage studied. There appeared to be a combination of a number of independent characteristics which made the products at this stage most desirable.

(c) The two varieties of field corn which were canned in the same manner as the sweet varieties were judged to be much inferior to the sweet sorts at comparable stages. In view of the greater moisture content, the hardness and toughness of the kernels, the poorer flavor, and the difference in the nature of the polysaccharides, there would seem to be little possibility of making a satisfactory canned product from these by any modification of the usual methods.

10. It appears that a first-quality canned product can be made from any sweet variety if the canning is done when the corn is at the proper stage of maturity.

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# THE PHOTOPERIODISM OF *TEPHROSIA CANDIDA*<sup>1</sup>

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## INTRODUCTION

Garner and Allard<sup>2</sup> through their observations and discussions on the effect of length of day on plant growth have opened a new and broad field for investigation.

Variation in the duration of exposure to daylight would seemingly have small influence on plant growth in the Tropics, where the range in day length is much more restricted than it is in the temperate regions. Such, however, is not the case, any change in day length being sufficient to exert a marked influence on plant growth. A knowledge of this influence as a factor in the growth of plants is of relatively greater importance in the Tropics, where plantings may be made every day in the year, than it is in the temperate regions where, at least in the higher latitudes, the planting season is more or less narrowly restricted by temperature.

The observations recorded in this paper were made at Mayaguez, P. R., which is little less than one-fourth degree north of the eighteenth parallel of north latitude. As is graphically shown in figure 1,<sup>3</sup> the time between sunrise and sunset in Mayaguez ranges from 11 hours in December to 13.2 hours in June.

## PRELIMINARY OBSERVATIONS

*Tephrosia candida*, a tropical legume which is used as a cover crop, was selected for observation of the effect of variation in length of day on blossoming and growth. *T. candida* is a woody plant which attains a height of 9 to 11 feet. It makes a good stand for about 18 months, after which it gradually weakens and dies. In some instances, however, the plants outlive the second year.

Plantings were made in duplicate rows at intervals of 14 days between December 21, 1921, and December 20, 1922. The rows were uniformly 25 feet in length, and were numbered consecutively from 1 to 27 with the date of planting, each two rows that were planted on the same day bearing the same number. The plants were thinned to 50 to the row.

The inflorescence is terminal and at first looks like a tiny, green, erect tassel. As soon as the tassel appears, the plant is considered as having budded. An examination in the field of 100 plants, which were considerably older than those in the experimental rows, showed the presence of two budded plants on April 12, and again on May 9, 1922. Five plants were in blossom, or had developed young pods, and 10 were budded May 23; 6 bore young pods and 17 others were budded June 2. No further observations were made of these plants as budding was soon afterwards noticed in the experimental rows. Between June 13 and June 26, 108 plants were budded in the 8 rows of the four earliest plantings, which was over one-fourth of the total number of the plants in these rows. No buds were observed, however, in the younger rows. The budded plants were labeled and dated for observation. About a month later, it was observed that the

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<sup>1</sup> Received for publication Mar. 6, 1924.

<sup>2</sup> GARNER, W. W., and ALLARD, H. A.—EFFECT OF THE RELATIVE LENGTH OF DAY AND NIGHT AND OTHER FACTORS OF THE ENVIRONMENT ON GROWTH AND REPRODUCTION IN PLANTS. Jour. Agr. Research 18: 553-606, illus. 1920.

<sup>3</sup> Data furnished by the U. S. Dept. Agr., Weather Bureau, San Juan, P. R.



plants were making little or no progress toward flowering, the small tassels in some instances atrophying accompanied by a change toward vegetative growth in the nearest leaf axils.

Only four plants blossomed prior to September 15, while more than a thousand opened their first flowers between September 15 and October 15, mainly in the last 10 days of September and the first 10 days of October. In rows No. 1 to 10, inclusive, which were planted between December 21, 1921, and April 26, 1922, 99 per cent of the plants were in blossom by the end of October. Eighty-eight per cent of the plants were in blossom in rows No. 11, 71 per cent in rows No. 12, and 19 per cent in rows No. 13. Rows No. 14, which were planted June 21, 1922, and all later plantings, failed to blossom (Pl. 1, A and B). The height of the plants in the various rows at this time is graphically shown in figure 2, the plants in rows No. 1 to 13, inclusive, ranging from less than 3 to more than 8 feet in height.

Toward the close of October, comparatively few blossoms opened, and the plants bore numerous pods. Only 1 or 2 per cent of the plants bore blossoms from early November until the middle of January, at which time 3 or 4 per cent

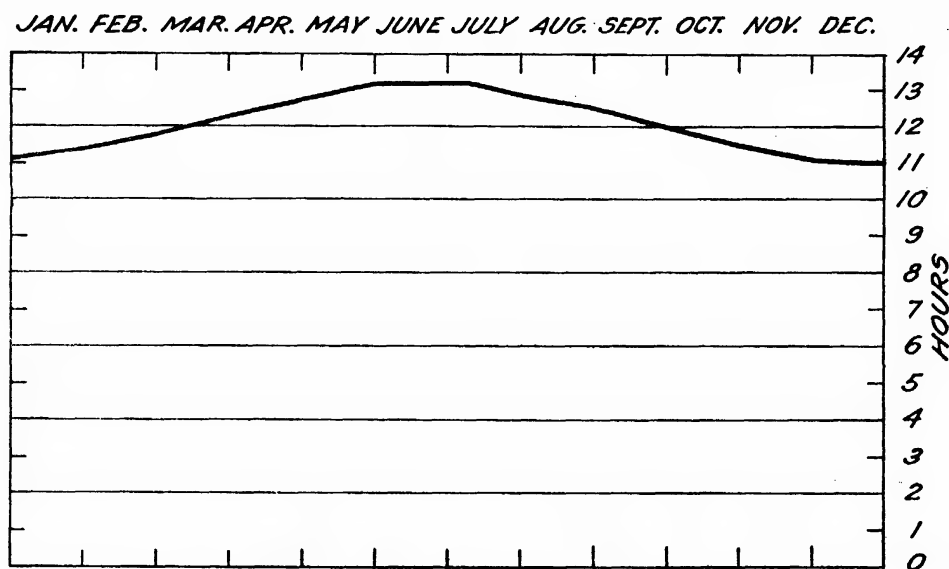


FIG. 1.—Length of day in hours, sunrise to sunset, Mayaguez, P. R.

flowered. Only three plants in the entire field blossomed in February. With the exception of one of these, all the plants blossoming in the winter had come into blossom in the autumn and continued to open a few scattered flowers now and then instead of setting heavy panicles of bloom. The only plant bearing flowers in March was one of the three which was seen with blossoms in February. The April record showed one, and the May record, two plants in blossom. Although only two plants were in flower June 20, 1923, many produced the tassel-like terminal tip of the young inflorescence. The tips later failed in most instances to produce flowers, and the plants resumed vegetative growth.

Plate 2, A, shows an atrophied inflorescence in the center of a representative specimen at the right, with vegetative growth well developed in the nearest leaf axils, and a number of shoots in varying stages (August 1, 1923). These shoots at first appeared as young inflorescence, but later instead of developing the terminal raceme or panicle of flowers, produced a stem of numerous short internodes wholly different from normal vegetative growth, followed or topped by normal growth of the stem and leaves (Pl. 2, B). By the end of August, about 6 per cent of the plants bore either flowers, or pods resulting from recent flowering, and about 54 per cent had budded.

It was noted that less than 2 per cent of the plants bore flowers on September 12, but that budding was very general. Approximately 5 per cent of the plants were in blossom by September 19, one-fourth of the total number were in blossom by September 26, and about one-half by October 3. Three-fourths of the total number were in flower or bore buds, which were about ready to open, by October 10. An examination of a half section of the field comprising 27 rows, one of the two rows planted on each planting date, showed that only about 8 per cent of the plants had failed to bloom by this time. Blossoming in the field was practically over by the end of the first week in November.

The blossoming record of the two seasons shows that, with a day length favorable to blossoming, practically all plants, except, of course, those weakened by age or disease, as old as 6 months (rows No. 10, planted April 26, 1922, the plants of which blossomed the following autumn), and a number only  $4\frac{1}{2}$  months old (rows No. 13, planted June 7, 1922), may be expected to bloom. The record also shows that plants that are much younger than  $4\frac{1}{2}$  months at the blossoming season will not only pass the first season without flowering, but will also not develop flowers until the return of the favorable day length the following year. This was exemplified by plants (rows No. 14 and higher) which, although planted June 21, 1922, and later, did not blossom until the autumn of 1923, when some were 15 and 16 months old. The results clearly show that the blossoming season of plants having reached a sufficient maturity is very definitely determined by the day length instead of by age.

The record further shows that (1) there is, in the course of a year, only one comparatively short and very definite season of heavy blossoming which reaches its height in midautumn; (2) this season is followed by a period covering several months during which a very few scattered flowers may open on plants that have already blossomed; (3) although there are indications of budding in the spring or early summer, such budding, except in some few instances, is not followed by flowers after a sufficient lapse of time; and (4) vegetative growth is resumed and in turn is followed by blossoming at the same season as in the preceding year.

#### EFFECT OF VARYING THE DAY LENGTH ON BLOSSOMING

In order to determine the effect on the plants of artificially protracting or shortening the length of daily exposure to light, a series of plantings was made in oil cans having a capacity of 5 gallons. The cans were uniformly filled with river-bottom loam. Three plants were grown to a can, two cans being planted with *Tephrosia candida* simultaneously with each field planting, and correspondingly numbered with the rows planted the same day. One of each pair of cans was lettered "A," and the other, "B." The plants in the A series were exposed to varying day lengths. Those in the B series were exposed to normal day length.

Since for the artificially shortened day the light exposure began abruptly, and for the artificially lengthened day terminated abruptly with the turning off of the electric light, a day artificially shortened or lengthened to 12 hours with only a single twilight exposure was shorter than the corresponding natural day of 12 hours between sunrise and sunset with its double twilight.

To give the plants additional light exposure, the cans were placed below a framework, 4 by 6 feet, carrying 6 tungsten filament incandescent lights, each rated at 25 candlepower, set 3 by 4 feet. An adjustable screen and black curtain, provided to furnish two distinct exposures, effectively protected half the space from the light used by the other half when two of the lights at one end were switched off. It being feared that the intensity of light was insufficient, the two middle lights were replaced by two of 60 candlepower at the end of the seventh week of lighting, and one at each end was replaced by one of 40 candlepower 9 days later.

The lights were switched on before sunset and off at the number of hours after sunrise requisite for making the desired light exposure or day length. All of the plants in the A series were given a 10-hour daily exposure at first. The day length was later modified in different ways until the autumnal equinox, 1923, from which time all the plants of the series were exposed to normal day length until the close of the period of observation, November 30, 1923. The following were the modifications made in the 10-hour daily period:

Plants in cans No. 1 to 18, inclusive, A series, were divided into groups 1, 2, and 3, November 10, 1922.

Group 1: Plants in cans No. 1, 4, 7, 10, 13, and 16 were subjected to a 10-hour day as before until March 14, 1923, when the day was lengthened to 12 hours.

Group 2: Plants in cans No. 2, 5, 8, 11, 14, and 17, were subjected to a 12½-hour day for 10 weeks, that is until January 19, 1923, and then to a 12-hour day.

Group 3: Plants in cans No. 3, 6, 9, 12, 15, and 18 were subjected to a 13½-hour day for 10 weeks, that is until January 19, 1923, and thereafter for 4 weeks to a 13-hour day, then for 2 weeks to a 12½-hour day, and finally, to a 12-hour day.

The plants in the remaining cans of the A series, No. 19 to 27, inclusive, were subjected to an 18-hour day length the first week after March 14, 1923, and the length was thereafter reduced by one-half hour weekly until May 17, after which it remained at 13½ hours for 2 weeks, and then was further reduced to 13.2 hours, the normal day length, for 1 week. After June 6, the cans of this lot were divided into groups 4 and 5.

Group 4: Plants in cans No. 20, 22, 24, and 26 were subjected to normal day length.

Group 5: Plants in cans No. 19, 21, 23, 25, and 27 were subjected to a 12-hour day length.

When subjected to the normal day length, the plants in cans flowered during both seasons a little later than did those in the field. Flowering and fruiting were normal, otherwise.

In cans No. 1 to 13, inclusive, B series, one or more plants came into blossom within a period of two weeks, October 11 to 25, 1922, and all but four which opened their first flowers in November had blossomed by the end of October. (Pl. 3, A.) Plants in cans No. 14 to 27, inclusive, B series, like those in the field tests, produced no flowers, all planted as late as June 21 failing to bloom.

The plants in cans No. 1 to 13, inclusive, A series, which had been given a 10-hour day throughout this period, showed no indication whatever of budding. (Pl. 3, B.)

By October, 1923, of the 39 plants in cans No. 1 to 13, inclusive, B series, 2 had died and 3 had made a very poor weak growth. Of the remaining 34 plants, 22 came into their second blossoming in October, and 6 in November.

The regularity in effect of the recurrent annual factor which induces blossoming may be noted by a comparison of the dates on which first blossoms appeared in cans No. 1 to 13, inclusive, B series, during two seasons, as shown in Table I.

Plants in cans No. 14 to 27, inclusive, B series, produced their first blossoms in October, 1923, when 31 of the 41 still living plants came into blossom. In November, 6 of the remaining 10 blossomed, 2 showed well-defined buds, 1 died budded, and 1 which had tasseled in October failed to develop.

That the interval between the first indication of budding, the appearance of the terminal tassel, and the opening of the flowers is closely related to the day length, was shown by observations made on the dates of the first budding and of the opening of the flowers. The plants in cans were carefully examined at weekly intervals for the first appearance of tassels. Table II gives the dates on which the tassels were first observed on 20 plants in cans No. 19 to 27, inclusive, B series, and the dates on which each opened its first flower.

TABLE I.—Comparison of dates of two seasons during which plants in cans No. 1 to 13, B series, opened their first blossoms

Can No.	October	
	1922	1923
1.....	17	16
2.....	21	24
3.....	18	18
4.....	17	19
5.....	25	24
6.....	23	25
7.....	14	16
8.....	15	(a)
9.....	22	31
10.....	14	16
11.....	11	18
12.....	19	24
13.....	16	15
Average dates of opening.....	18	20

<sup>a</sup> Date not noted, but was apparently within the preceding week, Oct. 16.

TABLE II.—Showing interval between first observance of budding and opening of flowers on 20 plants in cans No. 19 to 27, inclusive, B series

Date budded	Date flowered	Interval between budding and flowering	Average interval between budding and flowering
		Days	Days
Aug. 15.....	Oct. 9	55	52
Do.....	Oct. 10	56	
Aug. 22.....	Oct. 9	48	
Do.....	Oct. 13	52	
Aug. 29.....	Oct. 15	47	
Do.....	Oct. 16	48	46
Do.....	Oct. 23	55	
Sept. 5.....	Oct. 16	41	
Do.....	Oct. 18	43	
Do.....	Oct. 27	52	
Do.....	Oct. 28	53	31
Sept. 12.....	Oct. 26	44	
Do.....	Oct. 27	45	
Sept. 26.....	Oct. 23	27	
Do.....	Oct. 26	30	
Do.....	Oct. 27	31	21
Do.....	Oct. 30	34	
Oct. 3.....	Oct. 22	19	
Oct. 10.....	Oct. 31	21	
Do.....	Nov. 3	24	

The interval between the noting of budding and flowering averaged 52 days for plants budding in the last half of August, 46 days for those budding in the first half of September, 31 days for those budding in the last half of September, and only 21 days for those budding in the first half of October, when the day is 12 hours and less in length. As the days shortened, the interval proportionally shortened. Although there was a difference of 56 days between the earliest and latest dates on which the first buds were observed, all plants opened their first blossoms within a period of 25 days.

Groups 4 and 5, for which these cans were the controls, were subjected to a very long daily light exposure which was rapidly reduced. They did not differ in treatment until June 6, when the daily light exposure of group 5 was reduced to 12 hours. Group 4 was subjected to the normal day length, which remains at 13.2 hours from May 31 to July 11. As was true of the controls, group 4 showed

tassels in August and September, and opened first blossoms from September 23 to October 15. The August buds required on the average, 49 days to open, and the September buds, but 29 days. Group 5, however, with its artificially shortened 12-hour daily light exposure, showed tassels from June 27 to July 18, and opened first blossoms from July 20 to August 4, the interval between the first observance of tasseling and the date of blossoming averaging but 18 days.

If the autumn blossoming were merely the result in due time of the long summer days alone, group 4, which was subjected to an artificially lengthened day in spring merging into the long days of summer, would have been expected to blossom before the normal blossoming season. Four of the 12 plants in this group died before October, and the others opened their first blossoms between September 23 and October 15.

Group 5 was subjected to the same artificially lengthened day in spring, but the daily light exposure was shortened to 12 hours after June 6. The plants in two cans in this group failed to make vigorous growth, one dying of a root disease in August, and none blossoming. The remaining nine plants opened their first blossoms between July 20 and August 4, which was 7 to 9 weeks ahead of the earliest blossom in group 4.

The behavior of these two groups showed that blossoming was inhibited so long as exposure to the long days of summer continued, but that it was promptly induced by shortening the length of the daily exposure to 12 hours.

Plants in cans No. 1 to 18, inclusive, A series, were given a 10-hour day exposure at first, and all passed the autumn blossoming season, 1922, without flowering. The plants in 13 of the cans were of sufficient age to blossom, however, and their controls did blossom. Blossoming had been completely inhibited by a 10-hour day length.

In November, these 18 cans were divided into three groups, group 1 continuing with a 10-hour day, group 2 receiving a  $12\frac{1}{2}$ -hour day, and group 3 a  $13\frac{1}{2}$ -hour day for 10 weeks, at the end of which time budding was not evident. The artificially lengthened day was then shortened by one-half hour for each group. After four weeks, it was again shortened by half an hour for group 3. Two weeks later it was brought to a 12-hour length, where it was maintained for both groups.

A week after the first reduction in day length, budding appeared to be starting. A week later, budding was noted on 7 of the 14 plants in group 3 (1 in can No. 12, and 3 in can No. 15 having died), which had been subjected to the longer day, and on 1 of the 16 plants in group 2. With the exception of 2 weak, poor plants in can No. 3, which had been kept the longest of its group at a 10-hour day, and 1 plant which died budded in April (can No. 6), all the plants in group 3 opened their first blossoms between April 4 and April 27. (Pl. 4, A.)

Group 2, which had received only a  $12\frac{1}{2}$ -hour light exposure as its longest day, showed a much less pronounced tendency to come into blossom than did group 3. The first plant opened its first flower March 31, and 3 plants came into blossom in May, and 1 in each of the months of June, July, and August. Of the remainder, 7 died between May and October, and none flowered.

The light exposure of group 1 was changed on March 14 from 10 to 12 hours. Two plants came into blossom in July, and 2 in August, but the others failed to blossom.

From the behavior of these plants under varying day lengths, it is concluded that a day length of 10 hours is too short, while one equaling that of a June day in this latitude, 13.2 hours, is too long, for flowering, and that an intermediate day length is necessary to induce blossoming. While blossoming may occur under a suitable day length, whether the preceding days have been too long

or too short, the impulse to blossom is much more pronounced when the preceding days have been long rather than short, long days followed by shorter days quickly bringing into expression the reproductive process.

The explanation of the phenomenon of spring budding not being followed by flowering is that the reproductive stage is initiated by the spring days of favorable length, but that its further development is inhibited by the longer days of summer which induce a return to vegetative development. The starting and then the arresting of the reproductive stage, followed by the resumption of vegetative growth, are especially interesting in the Tropics, where winter cold cannot be considered as a possible factor in the arrested development of the reproductive process.

The growth of the plants is decidedly affected by the day length.

In figure 2, the height of the plants in the various rows in the field is shown for three different dates. The growth made during the short days from November to March, inclusive, amounted to very little for the plants in the older rows, rows No. 1 to 12, inclusive, increasing less than a foot in height, while the growth of the plants in the younger rows was much less than that made in the following four

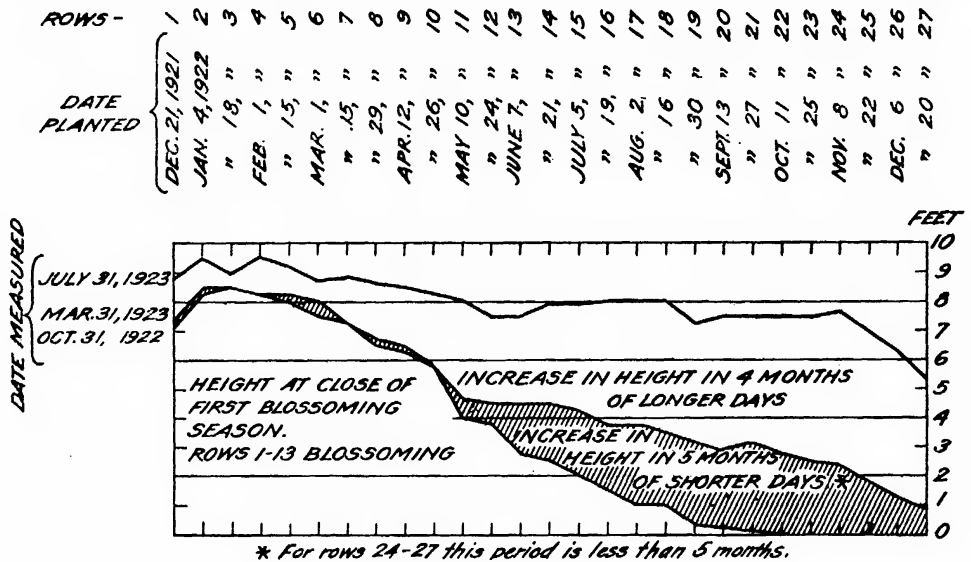


FIG. 2.—Growth in field

months with longer days. As the rainfall in this latter period is much more plentiful than it is in the earlier period, moisture, as well as day length, doubtless accounted for the increased growth. The cans were protected at this time from rainfall, but were well and frequently watered at all seasons. The height of the plants in cans No. 1 to 13, inclusive, B series, averaged 28.9 inches at 12 weeks from planting, while the correspondingly numbered plants, which were kept at a 10-hour daily exposure, averaged only 19.1 inches. The same plants at 24 weeks average 63.4 and 44.1 inches, respectively, in height.

Plants in cans No. 1 to 13, inclusive, B series, were measured at 12 weeks from planting. With the exception of one, all the plantings made between December 21 and April 12 and measured between March 15 and July 5, showed a steady increase in height (fig. 3). The day length began to shorten before the middle of July. Plants attaining the age of 12 weeks after July 15 showed a decrease in height, which was probably attributable to the inception of the reproductive stage. Measurements of height at 24 weeks showed the tallest growth on plantings of late March and early April, measured just prior to the blossoming season (fig. 4). The maximum difference in height between controls and treated lots was

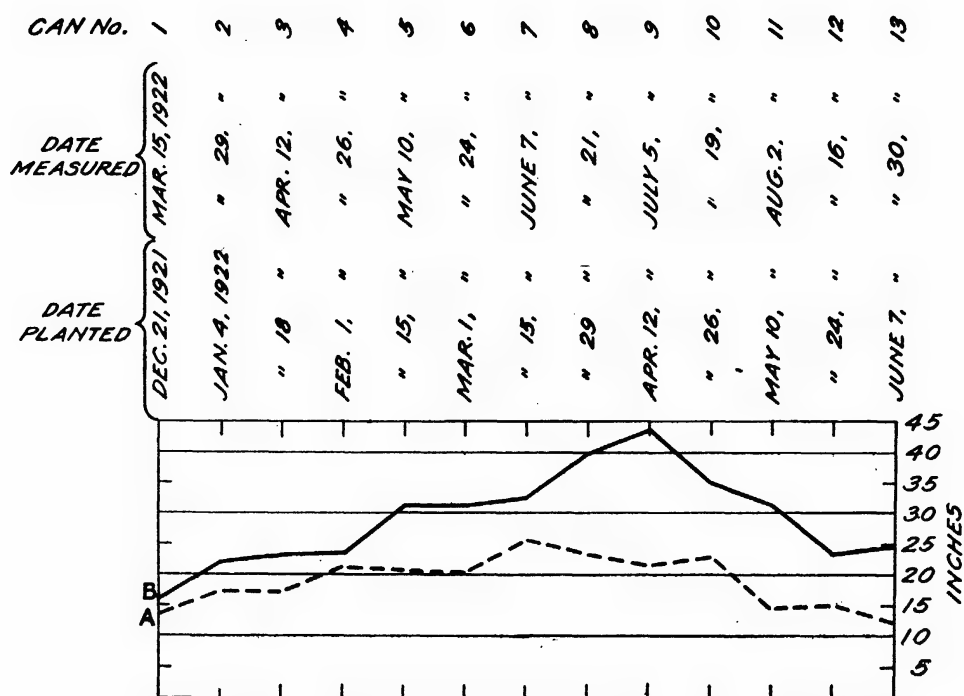


FIG. 3.—Average height per plant, cans No. 1 to 13, A and B series, at 12 weeks from planting. A, day length reduced to 10 hours. B, normal day

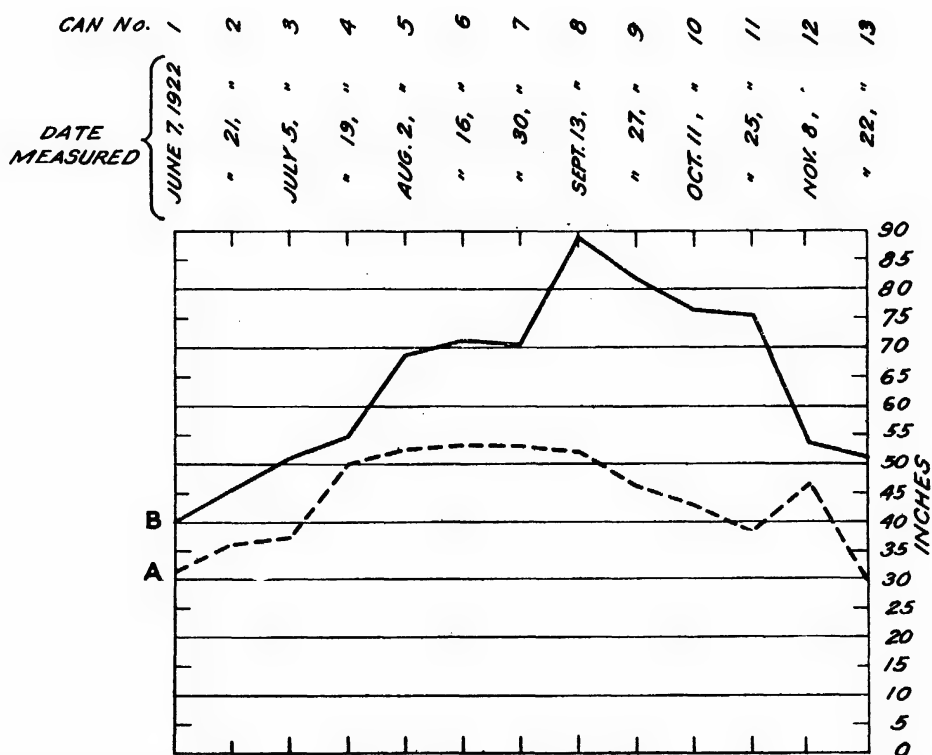


FIG. 4.—Average height per plant, cans No. 1 to 13, A and B series, at 24 weeks from planting. A, day length reduced to 10 hours. B, normal day

shown by plants in cans No. 8, which were planted March 29 and measured September 13, the B series measuring 82½, 89, and 93 inches in height, and the A series, 49½, 53½, and 54 inches. (Pl. 4, B.) These measurements showed individually and collectively that a reduction of day length to 10 hours had greatly retarded apogeotropic growth.

The opposite effect, achieved by lengthening the days, was shown by the plantings of August 16 in cans No. 18. Those of the A series were subjected to a 10-hour daily exposure for a little over 12 weeks after planting, that is until November 10. At this time they averaged 12.7 inches in height, while the control, with exposure to normal daily length, averaged 15.1 inches in height. The plants in the A series were subjected to a daily exposure of 13½ hours during 10 weeks, from November 10 to January 19, at the end of which time they averaged 52.6 inches in height, while the control, with exposure to normal day length, averaged only 35.9 inches. The growth of these plants up to a little more than 24 weeks from planting is graphically shown in figure 5.

EFFECT OF DAY LENGTH ON INTERNODAL LENGTH AND LEAF DIMENSIONS.

In order to determine the effect of day length on internodal length and leaf dimensions, the plants in cans No. 1 to 24, inclusive, were divided into four groups, cans No. 1 to 6, 7 to 12, 13 to 18, and 19 to 24, respectively. Each group was measured on the date on which its plants ranged from 12 to 22 weeks from planting. Prior to the taking of these measurements, the plants in the A series had uniformly received a 10-hour day length, and those in the B series, the normal day length. The tallest plant in each can was selected for measurement. Its height to the uppermost expanded leaf, number of nodes, and internodal length, are given in Table III.

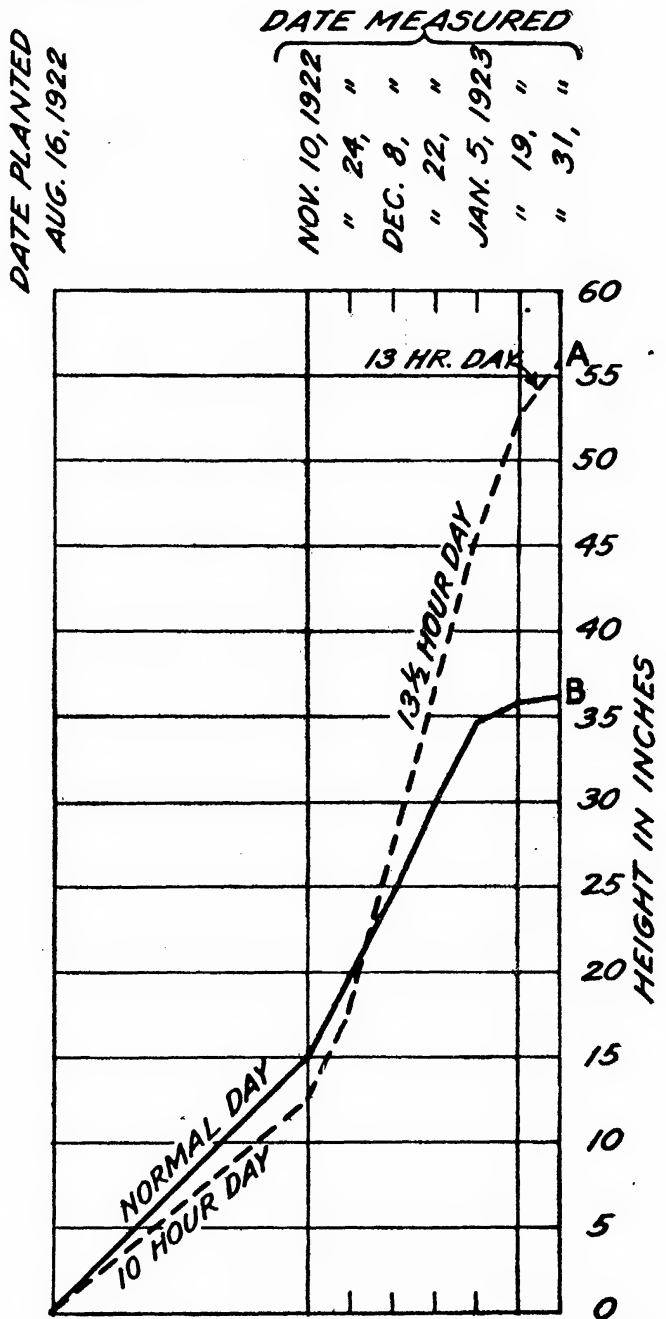


FIG. 5.—Average increase in height made by 3 plants each in cans No. 18, A and B series, under different light exposures.



TABLE III.—*Showing height and internodal length of tallest plants in cans No. 1 to 24, A and B series*

Date measured	Can No.	Date planted	Age of plantings when measured	Height to uppermost expanded leaf		Number of internodes included		Average internodal length, with stem below cotyledons considered as an internode	
				A	B	A	B	A	B
		1921	Weeks	Inches	Inches			Inches	Inches
	1	Dec. 21	22	27½	32	21	17	1.3	1.9
		1922							
May 24, 1922	2	Jan. 4	20	26½	36	19	19	1.4	1.9
	3	Jan. 18	18	24½	30	19	14	1.3	2.1
	4	Feb. 1	16	26½	31	15	14	1.8	2.2
	5	Feb. 15	14	24½	33½	15	14	1.6	2.4
	6	Mar. 1	12	18	28½	11	13	1.6	2.2
Total				147½	191½	100	91	9.0	12.7
Average				24.5	31.9	16.7	15.2	1.5	2.1
Aug. 16, 1922	7	Mar. 15	22	54½	65	30	29	1.8	2.2
	8	Mar. 29	20	48	71½	25	28	1.9	2.6
	9	Apr. 12	18	35	67¾	22	24	1.6	2.8
	10	Apr. 26	16	31½	46½	19	20	1.7	2.3
	11	May 10	14	16	38	12	15	1.3	2.5
	12	May 24	12	13½	23	10	12	1.4	1.9
Total				198½	311¾	118	128	9.7	14.3
Average				33.1	52	19.7	21.3	1.6	2.4
Nov. 8, 1922	13	June 7	22	33	55½	22	25	1.5	2.2
	14	June 21	20	28½	54	21	24	1.4	2.3
	15	July 5	18	26¾	44	18	24	1.5	1.8
	16	July 19	16	19½	42½	15	20	1.3	2.1
	17	Aug. 2	14	15½	28½	13	15	1.2	1.9
	18	Aug. 16	12	10¾	14¾	11	12	1.0	1.2
Total				133½	239½	100	120	7.9	11.5
Average				22.2	39.9	16.7	20	1.3	1.9
Jan. 31, 1923	19	Aug. 30	22	48	46¾	23	25	2.1	1.9
	20	Sept. 13	20	43	42½	22	21	2.0	2.0
	21	Sept. 27	18	33	33¾	19	18	1.7	1.9
	22	Oct. 11	16	30½	32½	17	16	1.8	2.0
	23	Oct. 25	14	24¾	28½	13	14	1.9	2.0
	24	Nov. 8	12	23	21¾	10	11	2.3	2.0
Total				202½	205½	104	105	11.8	11.8
Average				33.7	34.3	17.3	17.5	2.0	2.0

The fifth expanded leaf below the apex was arbitrarily chosen for leaf dimensions. Determination was made of the length of its midrib, number of pinnae, length of the terminal pinna, and also of one of the pair of pinnae midway of the leaf. The results are given in Table IV.

TABLE IV.—*Leaf measurements on tallest plant, fifth expanded leaf below apex cans No. 1 to 24, inclusive, A and B series*

Date measured	Can No.	Date planted	Age of plantings when measured	Length of midrib		Number of pinnae		Length of pinna mid-way of leaf		Length of terminal pinna	
				A	B	A	B	A	B	A	B
May 24, 1922.....	1	1921 Dec. 21	Weeks 22	Inches 4 $\frac{1}{2}$	Inches 6 $\frac{3}{4}$	Inches 21	Inches 21	Inches 1 $\frac{1}{2}$	Inches 2 $\frac{3}{8}$	Inches 2 $\frac{1}{4}$	Inches 2 $\frac{3}{8}$
	2	1922 Jan. 4	20	4 $\frac{5}{8}$	7 $\frac{5}{8}$	21	19	2 $\frac{1}{4}$	3 $\frac{1}{2}$	2 $\frac{3}{8}$	3 $\frac{1}{2}$
	3	Jan. 18	18	5	8	23	17	2	2 $\frac{1}{2}$	2	2 $\frac{1}{4}$
	4	Feb. 1	16	5 $\frac{1}{2}$	6 $\frac{1}{2}$	15	15	2 $\frac{3}{8}$	2 $\frac{3}{8}$	2 $\frac{3}{8}$	2 $\frac{3}{8}$
	5	Feb. 15	14	6	7 $\frac{1}{2}$	17	17	2 $\frac{1}{2}$	3 $\frac{1}{8}$	2 $\frac{3}{8}$	2 $\frac{3}{8}$
	6	Mar. 1	12	4 $\frac{1}{2}$	6 $\frac{3}{4}$	9	11	2 $\frac{3}{8}$	3 $\frac{1}{2}$	2 $\frac{3}{8}$	3 $\frac{1}{8}$
Total.....				29 $\frac{1}{2}$	42 $\frac{3}{4}$	106	100	13 $\frac{5}{8}$	18 $\frac{3}{8}$	14 $\frac{1}{2}$	17 $\frac{1}{4}$
Average.....				4.9	7.1	17.7	16.7	2.3	3.1	2.4	2.9
Aug. 16, 1922.....	7	Mar. 15	22	6 $\frac{1}{2}$	8 $\frac{5}{8}$	21	23	2 $\frac{1}{2}$	2 $\frac{5}{8}$	2 $\frac{3}{8}$	2 $\frac{1}{2}$
	8	Mar. 29	20	5 $\frac{1}{2}$	8	21	21	2 $\frac{1}{2}$	2 $\frac{1}{2}$	2 $\frac{1}{2}$	2 $\frac{1}{2}$
	9	Apr. 12	18	5 $\frac{1}{2}$	8 $\frac{5}{8}$	19	23	2 $\frac{1}{2}$	3 $\frac{1}{2}$	2 $\frac{1}{2}$	3 $\frac{1}{2}$
	10	Apr. 26	16	6	8 $\frac{1}{2}$	19	23	2 $\frac{3}{4}$	2 $\frac{3}{4}$	2 $\frac{3}{8}$	2 $\frac{1}{2}$
	11	May 10	14	3 $\frac{3}{4}$	6 $\frac{1}{2}$	11	17	2 $\frac{1}{4}$	5	2 $\frac{3}{8}$	3
	12	May 24	12	2 $\frac{1}{2}$	4 $\frac{5}{8}$	7	11	1 $\frac{1}{8}$	2 $\frac{1}{2}$	1 $\frac{1}{4}$	2 $\frac{1}{8}$
Total.....				29 $\frac{1}{2}$	44 $\frac{1}{2}$	98	118	13 $\frac{3}{8}$	17 $\frac{1}{4}$	14 $\frac{1}{2}$	16 $\frac{3}{4}$
Average.....				4.9	7.4	16.3	19.7	2.3	2.9	2.4	2.8
Nov. 8, 1922.....	13	June 7	22	5 $\frac{1}{2}$	5 $\frac{1}{2}$	17	21	2 $\frac{1}{4}$	2 $\frac{3}{8}$	2 $\frac{1}{2}$	2 $\frac{1}{2}$
	14	June 21	20	4 $\frac{1}{2}$	6 $\frac{5}{8}$	19	21	1 $\frac{1}{2}$	2 $\frac{1}{2}$	1 $\frac{1}{2}$	2 $\frac{1}{2}$
	15	July 5	18	6 $\frac{1}{2}$	8 $\frac{1}{2}$	17	21	2 $\frac{1}{2}$	2 $\frac{1}{2}$	2 $\frac{1}{2}$	2 $\frac{1}{2}$
	16	July 19	16	4 $\frac{3}{8}$	7 $\frac{3}{8}$	13	21	2 $\frac{1}{8}$	2 $\frac{1}{2}$	2 $\frac{1}{4}$	2 $\frac{1}{4}$
	17	Aug. 2	14	3	5 $\frac{5}{8}$	11	15	2	2 $\frac{1}{2}$	2 $\frac{1}{2}$	3
	18	Aug. 16	12	2 $\frac{1}{2}$	3 $\frac{3}{8}$	7	9	1 $\frac{1}{8}$	2	2 $\frac{1}{4}$	2 $\frac{1}{2}$
Total.....				25 $\frac{1}{2}$	36 $\frac{1}{8}$	84	108	12 $\frac{1}{4}$	15	13 $\frac{3}{8}$	15 $\frac{1}{8}$
Average.....				4.2	6	14	18	2	2.5	2.2	2.5
Jan. 31, 1923.....	19	Aug. 30	22	6 $\frac{1}{2}$	5 $\frac{1}{2}$	17	19	2 $\frac{3}{4}$	2 $\frac{1}{2}$	2 $\frac{1}{2}$	2 $\frac{3}{8}$
	20	Sept. 13	20	7 $\frac{1}{2}$	7 $\frac{1}{2}$	19	21	3	2 $\frac{3}{8}$	2 $\frac{1}{2}$	2 $\frac{1}{2}$
	21	Sept. 27	18	6 $\frac{1}{2}$	7 $\frac{3}{8}$	17	19	2 $\frac{1}{2}$	2 $\frac{1}{2}$	2 $\frac{1}{2}$	2 $\frac{1}{2}$
	22	Oct. 11	16	6 $\frac{1}{2}$	6 $\frac{5}{8}$	17	17	2 $\frac{1}{2}$	2 $\frac{1}{2}$	2 $\frac{1}{2}$	2 $\frac{1}{2}$
	23	Oct. 25	14	4 $\frac{3}{8}$	5 $\frac{1}{2}$	11	13	2 $\frac{1}{2}$	3 $\frac{1}{2}$	3 $\frac{1}{2}$	3 $\frac{1}{2}$
	24	Nov. 8	12	3 $\frac{1}{2}$	4 $\frac{1}{2}$	7	9	2	2 $\frac{3}{8}$	2 $\frac{1}{2}$	2 $\frac{1}{2}$
Total.....				33 $\frac{3}{4}$	36 $\frac{7}{8}$	88	98	15 $\frac{1}{4}$	16 $\frac{1}{8}$	16 $\frac{1}{2}$	16 $\frac{1}{2}$
Average.....				5.6	6.1	14.7	16.3	2.6	2.7	2.8	2.7

\* The pinna which should have been measured here was damaged. Entry is mean of that of leaf above and below.

Series B was greater than series A in height in inches to the uppermost expanded leaf in each instance in cans No. 1 to 18, inclusive, the difference being especially noticeable in the measurements of August 16 on plantings made from March 15 to May 24, and in the measurements of November 8 on plantings made from June 7 to August 16. In other words, the differences in height were pronounced on plantings which were made between March 15 and August 16, and on growth made up to early November (fig. 6). The greatest growth of any of the plants measured August 16 was that made during the period of longest days (the range from April 27 to August 15 being between 12.8 and 13.2 hours). In the measurements made January 31 on plantings of August 30 to November 8, and on growth made during the period of shortest days (the range from November 2 to January 31 being between 11 and 11.4 hours), the difference in height was found to be insignificant between the plants receiving a 10-hour daily length and their controls which were subjected to the normal day length.

The variations in the growth of the plants receiving a 10-hour day length would seem to indicate that, in addition to length of day, some other factor or factors influenced growth, such as difference in atmospheric moisture or temperature at the different seasons, or difference in the quality of sunlight, the sun being much higher in the sky at the hour at which the plants were brought from the dark chamber in summer.

Two factors account for the difference in height, namely, length of internode and number of nodes. In 22 of the 24 instances, the internodal length of the control plant exceeded that of the treated. In the graph illustrating this (fig. 7), the lines do not cross except in the last group, the measurements being for the period of shortest day length. That the difference was very pronounced for growth made in the period of greater day length was demonstrated by the growth of plants in cans No. 1 to 18, inclusive. In the case of plantings in cans No. 8

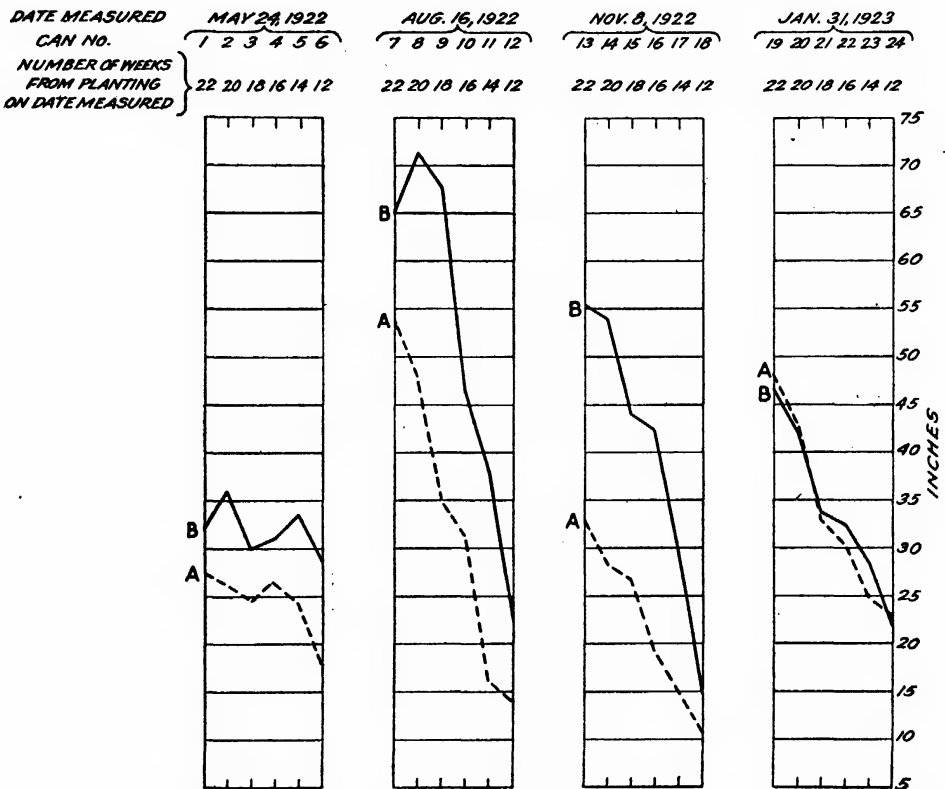


FIG. 6.—Height of tallest plant in cans No. 1 to 24, A and B series. A, day length reduced to 10 hours B, normal days

to 18, inclusive, made between the last of March and the middle of August, the control in each instance had more nodes than the treated plant, the average difference between control and treated being 2.8 nodes per plant. The others varied, their total measurements averaging only 0.7 node in difference between control and treated. In leaf measurements, the midrib of plants in the B series was longer than was true in case of the A series in 23 of the 24 instances, plants in can No. 19 being the exception.

Measurements were made of the fifth expanded leaf below the apex, this leaf presumably having developed, in most instances, approximately 4 to 6 weeks previously. Measurements of the midrib, made in May and August, showed a difference in length of 46 and 53 per cent, respectively, between the A and the B series. In the November measurement this difference was a trifle less pronounced amounting to 42 per cent. In the January measurements of leaves developed in the period of shortest days, this difference fell to 8 per cent.

That the number of pinnae also was affected by the day length would seem to be indicated in the count of pinnae of the plants in the A and the B series. In two instances, those of the A series had the greater number, in 5 the count was equal, and in 17 instances, plants of the B series had a greater number of pinnae than did those of the A series. The B series, with a normal day length, had a total of 13 per cent more pinnae than the A series, grown in a day of 10-hour length. This, however, was not borne out in measurements made January 19, and discussed later. (See Table IV.)

The length of the pinnae was decidedly affected by the day length. In length of pinna midway of leaf, the B series exceeded the A series in 21 of the 24 instances, equaled it in one, and was less than it in only two, these latter being in case of leaves which were measured January 31 and which had developed in the time of shortest day length. In the May, August, and November measurements, the difference between the A and the B series amounted to 37, 28, and 22 per cent, respectively, but in the January measurements this fell to 2 per cent. The difference in length of terminal pinna between the A series and the B series was

DATE MEASURED	MAY 24, 1922						AUG. 16, 1922						NOV. 8, 1922						JAN. 31, 1923					
CAN. NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
NUMBER OF WEEKS FROM PLANTING ON DATE MEASURED	22	20	18	16	14	12	22	20	18	16	14	12	22	20	18	16	14	12	22	20	18	16	14	12

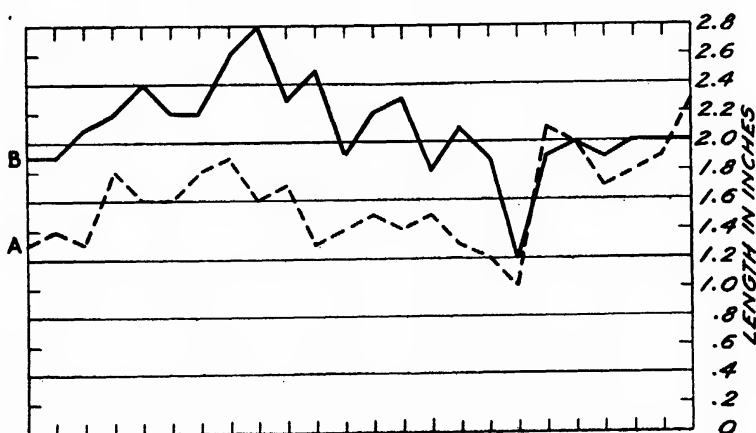


FIG. 7.—Average internodal length of tallest plant in cans No. 1 to 24, A and B series. A, day length reduced to 10 hours. B, normal day

decided, but not so pronounced as in the pinnae midway of the leaf. The B series exceeded the A series in 15 of the 24 instances, equaled it in 5, and was exceeded by the A series in only 4 instances, one of which was an estimated measurement in lieu of that of a damaged pinna, for which the mean of the leaves above and below was taken. The differences for the May, August, and November measurements amounted to 18, 19, and 13 per cent, respectively, while in the January measurements this fell to 2 per cent, as in the case of the midway pinna.

Of the controls for groups 1, 2, and 3, the plants in all cans to No. 13, inclusive, flowered in the autumn of 1922, for the time discontinuing further vegetative growth. On January 19, 1923, leaf measurements were made on all plants of which the controls in these three groups had not flowered, namely the plants in cans No. 14 to 18, inclusive, and on their controls. During the 10 weeks preceding this date, can No. 16 had received a day artificially shortened to 10 hours, cans No. 14 and 17, a day artificially lengthened to 12½ hours, and cans No. 15 and 18, all A series, a day lengthened to 13½ hours. In this period of 10 weeks, the normal day had shortened from 11.4 hours to 11 hours, and then lengthened to 11.2 hours.

In order to measure a leaf which would show as fully as possible the effect of the 10-week lighting period and at the same time be well-developed, the second expanded leaf below the apex was measured on each plant, and also the length of stem from the node below this leaf to the node above it. That the day length very decidedly affected internodal length is indicated by the data presented in Table V.

TABLE V.—Internodal length produced under daily light exposures of 10 to 13½ hours for A series, and normal day length (11 to 11.2 hours), for B series<sup>a</sup>

Can No.	Plant No.	Length of daily light exposure	Combined length of two internodes below uppermost expanded leaf	
			A	B
		Hours	Inches	Inches
16.....	{ 1 2 3 }	10	{ 2½ 3½ 2½ }	{ 2½ 1½ 2½ }
Total.....			8	6½
Average internodal length.....			1.3	1.1
14.....	{ 1 2 3 }	12½	{ 4½ 5½ 5½ }	{ 2½ 1½ (b) 1½ }
17.....	{ 1 2 3 }	12½	{ 6½ 4½ (b) 4½ }	{ 1½ 2½ 3½ }
Total.....			26	11
Total internodal length.....			2.6	1.1
15.....	{ 1 2 3 }	13½	{ 6½ 7½ (b) 7½ }	{ 2½ 2½ 2½ }
18.....	{ 1 2 3 }	13½	{ 7½ 6 8 }	{ 3½ 3½ 5½ }
Total.....			35½	19½
Average internodal length.....			3.5	1.6

<sup>a</sup> Measured Jan. 19, 1923.

<sup>b</sup> This plant was lacking.

Since the plants were few in number, the figures should not be too closely interpreted. The greatest average internodal length for growth made by any group receiving normal day length amounted only to 1.6 inches, while under a 12½-hour day length, it amounted to 2.6 inches, and under a 13½-hour day length, to 3.5 inches. Although growth made under the 10-hour day length exceeded its own control, it fell within the range of the three controls. The longer days produced longer internodes.

The effect of day length on midrib length was most pronounced, as shown by Table VI.

Under daily light exposures of 10, 12½, and 13½ hours, the average midrib lengths were 5, 7, and 9 inches, respectively, while the average for their controls ranged from 4.6 to 5.5 inches. With the exception of one dying plant, every individual in the 13½-hour group exceeded in length of midrib every plant in the 12½-hour group, each of which in turn exceeded each of the plants of the 10-hour group. The longer days produced leaves with longer midribs.

The indication seen in the earlier measurements of the development of a greater number of pinnae in the longer day was not borne out by the count on these leaves. However, the number of plants was small. The treated plants averaged 19 pinnae for each group, while 2 controls averaged 20, and the third control, 21.

The effect of day length on pinna length was most pronounced, as shown by Table VII.

TABLE VI.—Length of midrib of leaf as affected by daily light exposures of 10 to 13½ hours for A series, and normal day length (11 to 11.2 hours) for B series<sup>a</sup>

Can No.	Plant No.	Length of daily light exposure	Length of midrib of second expanded leaf below apex	
			A	B
		Hours	Inches	Inches
16.....	{ 1 2 3 }	10	{ 4½ 5 5½ }	{ 4½ 3½ 5½ }
Total.....			15	13½
Average.....			5	4.6
14.....	{ 1 2 3 }	12½	{ 6½ 6½ 6½ }	{ 4½ 4½ 4½ }
17.....	{ 1 2 3 }	12½	{ 8½ 7½ (b) }	{ 4½ 4½ 6½ }
Total.....			34½	23½
Average.....			7	4.7
15.....	{ 1 2 3 }	13½	{ 9 c 7½ (b) }	{ 5½ 4½ 4½ }
18.....	{ 1 2 3 }	13½	{ 9½ 10 9½ }	{ 5½ 5½ 7½ }
Total.....			44½	33½
Average.....			9	5.5

<sup>a</sup> Measured Jan. 19, 1923.<sup>b</sup> Plant was lacking.<sup>c</sup> Plant had wilted and was dying of a root disease when measured.TABLE VII.—Length of pinnae as affected by daily light exposures of 10 to 13½ hours for A series, and normal day length (11 to 11.2 hours) for B series<sup>a</sup>

Can No.	Plant No.	Length of daily light exposure	Length of pinna midway of leaf		Length of terminal pinna	
			A	B	A	B
		Hours	Inches	Inches	Inches	Inches
16.....	{ 1 2 3 }	10	{ 1½ 2 1½ }	{ 1½ 1½ 1½ }	{ 2½ 2½ 1½ }	{ 2½ 1½ 1½ }
Total.....			5½	5½	6½	5½
Average.....			1.9	1.8	2.1	2.0
14.....	{ 1 2 3 }	12½	{ 2½ 2½ 2½ }	{ 1½ 1½ (b) }	{ 2½ 2½ 2½ }	{ 1½ 1½ (b) }
17.....	{ 1 2 3 }	12½	{ 2½ 2½ (b) }	{ 2 2½ 2½ }	{ 2½ 2½ (b) }	{ 2½ 2½ 2½ }
Total.....			13½	9½	12½	10½
Average.....			2.7	1.9	2.5	2.1
15.....	{ 1 2 3 }	13½	{ 3½ 3½ (b) }	{ 2½ 2½ 2½ }	{ c 2½ 2½ (b) }	{ 2½ 1½ 2 }
18.....	{ 1 2 3 }	13½	{ 3½ 3½ 3½ }	{ 2½ 2½ 2½ }	{ 2½ 2½ 2½ }	{ 2½ 2½ 2½ }
Total.....			17½	13	13½	13½
Average.....			3.5	2.2	2.8	2.3

<sup>a</sup> Measured Jan. 19, 1923.<sup>b</sup> This plant was lacking.<sup>c</sup> This pinna was diseased. The corresponding one on leaves above and below measured 3 and 3½ inches, respectively.

The pinnae midway of the leaves measured averaged in length for daily exposures of 10, 12½, and 13½ hours, 1.9, 2.7, and 3.5 inches, respectively, while the controls exposed to a normal short day, averaged in length from 1.8 to 2.2 inches. Similarly, but in a less pronounced degree, the terminal pinna was affected, the average lengths for the 10, 12½, and 13½ hour exposures being 2.1, 2.5, and 2.8 inches, respectively, and for their controls ranging from 2 to 2.3 inches.

These measurements show very definitely that a day length of 12½ or 13½ hours is much more favorable to leaf development than a day length of 10 or 11 hours, the longer day causing the development of a much larger leaf, longer in both midrib and pinnae. These measurements on plants grown under an artificially shortened and an artificially lengthened day explain the pronounced difference in appearance between the plants in the field in late fall and winter, with their slow growth, short internodes, and small leaves, and the plants in late spring, with their rapid growth, long internodes, and large leaves. This contrast is shown in Plate 2, B, on a branch obtained from row No. 16, planted July 19, 1922, and photographed May 9, 1923.

### SUMMARY

The blossoming of *Tephrosia candida* was inhibited both by a day length shortened to 10 hours, and by a day length of 13.2 hours (length of the longest summer days in Mayaguez, P. R.).

Shortening the day from the long day of summer to a 12-hour length promptly induced blossoming, whereas a continuation of exposure to the long summer days inhibited it.

Under a day length artificially protracted to 13½ hours for 10 weeks, buds failed to appear, but on shortening the day length they promptly appeared.

Under exposure to a maximum day length of 12½ hours, later reduced to 12 hours, the plants displayed much less tendency to come into blossom than was the case when reduction was made from a longer day.

There was still less tendency to blossom when the day, instead of being shortened, was lengthened from 10 to 12 hours.

While blossoming may occur under a 12-hour day, whether the preceding days have been too short or too long for blossoming, the impulse to blossom apparently is much more pronounced when the preceding days have been too long rather than too short, days of too great a length followed by shorter days quickly inducing the reproductive stage.

Although autumn is the normal blossoming season for *T. candida*, heavy blossoming was induced in April through artificial manipulation of the length of light exposure or day length.

Day length not only determines the blossoming season for *T. candida*, but it also affects its growth in a pronounced and decided manner, the longer days producing growth with longer internodes and larger leaves.

# PLATE 1

A.—Simultaneous blossoming of rows up to No. 13 planted June 7 and earlier. No blossoms on later plantings. Photographed October 28, 1922.

B.—Another section of same field photographed on same date, showing rows No. 7 to 12 in simultaneous blossoming though planted at two-week intervals.







## PLATE 2

A.—Growth to be seen in midsummer, the reproductive stage having been started by the spring days of intermediate length, then arrested by the long days of summer, with a resumption of vegetative growth. Photographed August 1, 1923.

B.—Normal vegetative development, showing the short leafed, short internodal growth made in the shorter days, in contrast with the later growth of large leaves, and long internodes typical of growth made in the longer days. Photographed May 9, 1923.

### PLATE 3

A.—Growth and flowering under normal day length, all plants either budded, in blossom, or with young seed pods. Photographed October 27, 1922.

B.—Growth under a 10-hour day. No indication of blossoming. Planted on same dates as B plants correspondingly numbered. Photographed October 27, 1922.





#### PLATE 4

A.—Plants in can No. 18A, brought into blossom out of season by exposure to  $13\frac{1}{2}$ -hour day, followed by reduction in day length. Plants in cans No. 17A, which had received a  $12\frac{1}{2}$ -hour day and in No. 16A, a 10-hour day, and in B cans, a normal day length, all without buds or blossoms. Photographed April 19, 1923.

B.—Difference in height attributable to difference in length of day. Series A, grown under a 10-hour light exposure, and series B, under normal day length. Photographed August 18, 1922.

# ANTINEURITIC VITAMIN IN POULTRY FLESH AND EGGS<sup>1</sup>

By RALPH HOAGLAND, *Senior Biochemist, Biochemic Division*, and ALFRED R. LEE, *Poultryman, Animal Husbandry Division, Bureau of Animal Industry, United States Department of Agriculture*

## POULTRY AND EGGS IN THE DIETARY

The importance of vitamins in the human dietary is so well established that the nutritive value of a food product must now be judged not only by the usual standards but by its vitamin content as well. It does not follow, of course, that because a foodstuff is deficient in one or even all of the vitamins that it is of low nutritive value. Indeed, several of our most important foodstuffs are practically devoid of vitamins. And, on the other hand, a food product is not necessarily of high nutritive value solely because of its richness in one or more of the vitamins; but a food that is rich in vitamins, as well as having a high nutritive value in other respects, is greatly to be prized. What is needed, of course, is adequate information concerning the vitamin content of all our food products, so that each may be used in proper combination with others so as always to insure an abundance of each of the vitamins in the diet.

Poultry occupies an important place in the dietary of the American people, particularly on the farm, but it is highly regarded by all classes of people. Poultry flesh is generally considered to have as high nutritive value as beef, pork, or mutton. The per capita consumption of poultry in this country in 1919 is estimated as 11.5 pounds. For the same year, the estimated per capita consumption of beef and veal was 65.7 pounds; pork, excluding lard, 68.6 pounds, and mutton and lamb 5.8 pounds.

Eggs are one of our most important foods. Their high nutritive value is well established and no other food can quite take their place. Not only are they used extensively as such as a food, but they also enter into the preparation of a great variety of food products. According to the census the number of eggs produced on farms in the United States in 1919 was 1,654,044,932 dozens. For the same year exports and imports, including dried and frozen eggs, were practically equal. After making deductions for eggs used for hatching and for loss of eggs due to breakage and spoilage, it is estimated that the per capita consumption of eggs in this country in 1919 was 159.

## PREVIOUS INVESTIGATIONS

There seems to be no information in the literature concerning the vitamin content of poultry flesh, although it is possible that the report of some investigation may have been overlooked.

The vitamin B content of eggs has been studied by a number of investigators, some having determined the antineuritic value by means of feeding tests with

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<sup>1</sup> Received for publication March 17, 1924.



pigeons, while others have ascertained the value of eggs as a source of vitamin B for growth in young rats. Following are the opinions of several authorities concerning the value of eggs as a source of vitamin B:

McCarrison (4, p. 12).<sup>2</sup> "This vitamin is widely distributed throughout natural foodstuffs. Its richest sources are the germs of seeds, eggs, yeast, wheat and rice bran, peas, beans, lentils, and cellular organs (such as liver, brain, sweetbreads, fish-roe, kidneys, and heart muscle.)"

Sherman and Smith (6, p. 86). "Eggs. There is probably as high a concentration of vitamin B in the egg, or at least in the egg yolk, as in any part of the body. This is indicated both by the experiments of Cooper upon prevention of polyneuritis and those of Osborne and Mendel upon the support of growth. The latter results have apparently not yet been published in full, but are referred to in terms which would imply that the solids of eggs and of milk have about equal proportions of the water-soluble vitamin."

The work of Cooper (1) deserves special mention because it was carried on by methods very similar to those employed in the investigation that is to be reported in this paper. Cooper determined the antineuritic value of both raw and cooked egg yolk when fed to pigeons together with polished rice. Raw egg yolk was fed daily to groups of 2 pigeons each, in quantities varying from 1 to 10 gm. for each bird. It was found that when 5 gm. of raw egg yolk were fed daily to each of 2 pigeons that they did not develop polyneuritis during a period of 55 days, but they lost greatly in weight to the extent of 30.7 and 32.1 per cent, respectively. Ten grams of raw egg yolk in the daily ration of each of two pigeons protected them against polyneuritis during a period of 55 days, but they had lost 3 and 7.5 per cent, respectively, in weight.

Cooked egg yolk had a similar value to the raw product. Six grams of cooked egg yolk in the daily ration of each of 2 pigeons protected them against polyneuritis for 50 days, but they had lost 12.2 and 19 per cent, respectively, in weight.

In conclusion, Cooper, states:

Egg yolk exceeds all the other foodstuffs examined of animal origin in antineuritic value, three grams daily added to the rice diet being sufficient to prevent polyneuritis. Its capacity for preventing this disease is not measurably altered by boiling for four minutes.

Osborne and Mendel (5) report that—

Rats weighing 100 gm. living on a standard food mixture, of which they consumed about 50 gm. per week, have required not less than 0.8 gm. of dried egg yolk a day (equivalent to 10 per cent of this food mixture) fed apart from the rest of the ration, to secure even an approximation to the normal rate of growth; and in every case the rate of gain has increased when 0.2 gm. of dried brewers' yeast replaced the egg-yolk preparation.

In conclusion the authors state in part:

The content of egg yolk in vitamin B is not large, a daily intake of at least 1.5 gm. of the fresh yolk being required when it furnishes the sole source of vitamin B to a 100-gm. rat. The whole egg is accordingly not exceptionally rich in vitamin B when contrasted with the foods already investigated. Judged by the comparative trials on rats, the average sized hen's egg is equivalent to about 150 c. c. of cow's milk, or, a quart milk and six or seven eggs of the average sort have an approximately equivalent vitamin B value.

## EXPERIMENTAL WORK

The purpose of this investigation was to determine the vitamin B (antineuritic vitamin) content of dried poultry flesh and eggs as measured by the quantity of either product, required in a ration with polished rice to protect a pen of four pigeons against both development of polyneuritis and loss in weight during a test period of 56 days.

The methods employed are essentially the same as those previously employed by one of the writers (2,3) in a study of the antineuritic properties of the edible tissues of the ox, sheep, and hog. The feeding tests with pigeons were conducted by Abraham Balter, formerly laboratory aid in the Biochemic Division.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 472.

## DESCRIPTION OF POULTRY AND EGGS TESTED

## CHICKENS

Rhode Island Red, Barred Plymouth Rock, and White Leghorn chickens were hatched in April, 1922, out of good breeding stock. They were raised in grass fields where they had free range and secured an unlimited amount of green feed. They received practically the same feed from date of hatch until they were killed at approximately four months of age. As small chicks, they were first fed a mixture of boiled eggs and rolled oats with a commercial baby-chick scratch feed. At the age of two weeks the use of the egg-oatmeal mixture was discontinued and they were given a mash made up in parts by weight as follows:

4 parts rolled oats.	1 part middlings.
2½ parts meat scrap.	½ part ground bone
2 parts corn meal.	1 per cent salt.
2 parts bran.	

This mash was kept in hoppers before the chickens at all times until they were killed. As soon as they were large enough to eat ordinary grain, at about 8 weeks of age, the baby-chick scratch feed was omitted and a feed consisting of equal parts of cracked corn and wheat was substituted.

The Rhode Island Red and Barred Plymouth Rock chickens weighed about 3½ pounds each when killed, and the White Leghorns weighed about 2½ pounds. All the chickens in the three lots used were cockerels.

## HENS

Rhode Island Red, Barred Plymouth Rock, and White Leghorn hens were used in tests of the flesh of mature fowls. The average dressed weights of the hens in each of the three lots were 5, 5½, and 3½ pounds, respectively. Some of the hens were 1½ years old and others 2½ years old, but all had been given practically the same kinds of feeds throughout their lives. All had been fed the same scratch feed and but slightly different mashes, substantially as follow:

<i>Mash</i>	<i>Scratch Feed</i>
10 parts bran.	2 parts cracked corn.
10 parts middlings.	1 part wheat
10 parts ground oats.	1 part oats.
45 parts corn meal.	
25 parts meat scrap.	

The proportions of the several constituents, other than the meat scrap, varied somewhat in the different mashes, but all the feeds were used in each mash. Each lot of hens was kept in a good-sized yard where there was more or less green feed either in the form of grass or as a growing crop of young oats, wheat, or rape. In addition, each pen of hens was fed three times weekly as much sprouted oats as they would consume. Approximately equal parts of mash and scratch feed were fed daily.

## TURKEYS, DUCKS, AND GUINEA FOWL

The above-named birds were purchased in January, 1923, from a local produce dealer. They had been shipped alive from Maryland and Virginia farms and were killed at the time of purchase. The turkeys consisted of 4 fat hens weighing 11½, 11¾, 12, and 15½ pounds, respectively. The ducks consisted of 15 common farm ducks of fair quality. The guinea fowl were fair-quality birds weighing about 2½ pounds each, the lot containing 22 birds.

HENS' EGGS

The eggs used in the tests for vitamin B were laid by hens kept under similar conditions and fed the same feeds as those that were killed. The eggs were collected in September, 1922, when the hens were receiving a considerable quantity of green feed. At that time the hens were nearing the end of their laying season.

METHOD OF DRYING POULTRY FLESH AND EGGS

The flesh, fat, and skin of the frying chickens were ground together, but the muscle tissue of the hens and other birds was separated from the skin and from the fat, as far as practicable, before grinding. The ground flesh was thoroughly mixed with water and toluol in the proportion of 800 grams, 400 cubic centimeters, and 40 cubic centimeters, respectively, and the mass was spread out in shallow pans and dried in an oven in a current of air at a temperature that did not exceed 60° C. The tissue became dry in 18 to 20 hours, after which it was ground, transferred to bottles, and held in cold storage until needed.

The eggs were separated from the shells, thoroughly beaten, spread out in a thin layer in shallow pans, and dried in a current of air at a temperature of 60° C.

FEEDING TESTS WITH RATIONS VERY DEFICIENT IN ANTINEURITIC VITAMIN

In order to judge the antineuritic value of a ration containing the product to be tested, it is necessary to have for comparison the results of other experiments conducted in the same manner in which the pigeons have been fed rations practically devoid of antineuritic vitamin. In Table I are reported the results of a number of such tests previously reported by one of the writers (2, 3).

TABLE I.—Feeding tests with rations very deficient in antineuritic vitamin

Rations		Pigeon No.	Initial weight	Final weight	Change in weight	Survival period	Result
Ingredients	Per cent						
Pen 1:			Grams	Grams	Per cent	Days	
Autoclaved rice.....	100	2, 061	366	348	—4. 9	18	Polyneuritis.
		2, 062	362	284	—21. 5	18	Do.
		2, 064	316	255	—19. 3	28	Do.
		2, 065	425	303	—28. 7	25	Do.
Average.....			367	298	—18. 6	22	
Pen 2:							
Autoclaved rice.....	100	249	425	383	—9. 9	17	Polyneuritis.
		250	470	273	—41. 9	46	Do.
		251	389	282	—27. 5	24	Do.
		252	419	306	—27. 0	24	Do.
Average.....			426	311	—26. 6	28	
Pen 3:							
Autoclaved rice.....	76	2, 066	305	280	—8. 2	21	Polyneuritis.
Autoclaved beef.....	15	2, 067	362	320	—11. 6	21	Do.
Cod-liver oil.....	5	2, 068	304	256	—15. 8	27	Do.
Ash mixture.....	4	2, 069	300	225	—25. 0	29	Do.
Average.....			318	270	—15. 2	25	
Pen 4:							
Autoclaved rice.....	76	245	379	329	—13. 2	21	Polyneuritis.
Autoclaved beef.....	15	246	396	275	—30. 6	42	Do.
Butterfat.....	5	247	377	304	—19. 4	28	Do.
Ash mixture.....	4	248	335	311	—7. 2	21	Do.
Average.....			372	305	—17. 6	28	

From the data presented in Table I it will be noted that the average survival period of the pigeons in pens 1 and 2 receiving only autoclaved rice was 25 days, while the average survival period of the pigeons in pens 3 and 4 receiving rice supplemented with dried autoclaved beef, cod-liver oil or butterfat, and ash mixture was practically the same, or 26½ days. The average loss in weight of the birds in pens 3 and 4 was somewhat less than that of the pigeons in pens 1 and 2, being 16.4 per cent as compared with 22.6 per cent. The average survival period of all the pigeons in pens 1 to 4, inclusive, was 26 days, and the average loss in weight was 19.5 per cent.

ANTINEURITIC VITAMIN IN THE FLESH OF FRYING CHICKENS

In Table II are presented the results of feeding tests with the dried flesh of frying chickens of three breeds, viz, Rhode Island Red, Barred Plymouth Rock, and White Leghorn. Each lot of dried flesh was fed to 4 pens of 4 pigeons each, the proportions of flesh in the several rations being 5, 10, 25, and 30 per cent. The data in the table show that each of the three lots of poultry (fryers) flesh had a rather low antineuritic value. The rations containing 5 and 10 per cent respectively, had no higher antineuritic value than the check rations reported in Table I, while the addition of even 25 or 30 per cent of the poultry flesh to the other rations gave them only slightly higher values. In no case did a ration containing even 30 per cent of fryer flesh protect an entire pen of pigeons against polyneuritis for 56 days, the maximum average survival period being 34 days. Only one pigeon (No. 154, pen 8) out of the 48 birds reported in Table II survived the test period of 56 days, and this bird was much emaciated at the close, having lost 35.2 per cent in weight.

TABLE II.—Antineuritic value of flesh of frying chickens

Ration		Pigeon No.	Change in weight	Survival period	Result
Ingredients	Per cent				
Pen 5:			<i>Per cent</i>	<i>Days</i>	
Rhode Island Red, fryer flesh.	5	2080	−20.0	20	Polyneuritis. Extreme emaciation; experiment discontinued. Polyneuritis. Do.
		2081	−32.7	35	
Autoclaved rice.....	95	2082	−15.3	19	
		2083	−17.6	21	
Average.....			−21.4	24	
Pen 6:					
Rhode Island Red, fryer flesh.	10	2084	−26.4	17	Polyneuritis. Do. Do. Do.
		2085	−9.3	21	
Autoclaved rice.....	90	2086	−15.5	21	
		2087	−14.7	29	
Average.....			−16.5	22	
Pen 7:					
Rhode Island Red, fryer flesh.	25	148	−20.2	52	Died. Polyneuritis. Do. Died.
		149	−12.8	18	
Autoclaved rice.....	75	150	−16.9	30	
		151	−21.3	36	
Average.....			−17.8	34	
Pen 8:					
Rhode Island Red, fryer flesh.	30	152	−26.5	35	Polyneuritis. Died. Emaciated at end of test. Died.
		153	−22.6	29	
Autoclaved rice.....	70	154	−35.2	56	
		1259	−17.7	17	
Average.....			−25.5	34	
Pen 9:					
Barred Plymouth Rock fryer flesh.	5	2089	−18.8	17	Polyneuritis. Do. Do. Died.
		2091	−13.3	17	
Autoclaved rice.....	95	2092	−29.0	21	
		2093	−29.7	22	
Average.....			−22.7	19	

TABLE II.—Antineuritic value of flesh of frying chickens—Continued

Ration		Pigeon No.	Change in weight	Survival period	Result
Ingredients	Per cent				
Pen 10:					
Barred Plymouth Rock, fryer flesh.	10	2094	<i>Per cent</i> -38.0	<i>Days</i> 35	Extreme emaciation; experiment discontinued. Polyneuritis. Do. Do.
Autoclaved rice.....	90	2095	+4.5	14	
		2096	-5.7	13	
		2097	-21.3	24	
Average.....			-15.1	22	
Pen 11:					
Barred Plymouth Rock, fryer flesh.	25	156	-41.2	34	Died. Polyneuritis. Do. Do.
Autoclaved rice.....	75	157	-4.9	25	
		158	-20.7	26	
		159	-14.2	34	
Average.....			-20.3	30	
Pen 12:					
Barred Plymouth Rock, fryer flesh.	30	160	-12.4	28	Polyneuritis. Do. Died. Polyneuritis.
Autoclaved rice.....	70	161	(a)	24	
		162	-10.9	32	
		163	-15.1	30	
Average.....			-12.8	29	
Pen 13:					
White Leghorn, fryer flesh...	5	2057	-17.5	25	Polyneuritis. Died. Polyneuritis. Do.
Autoclaved rice.....	95	2058	-24.4	20	
		2059	-9.9	17	
		2060	-11.1	14	
Average.....			-15.7	19	
Pen 14:					
White Leghorn, fryer flesh...	10	2098	-9.6	22	Polyneuritis. Do. Do. Extreme emaciation; discontinued experiment.
Autoclaved rice.....	90	2099	-12.2	17	
		2100	-21.5	17	
		190	-37.3	35	
Average.....			-20.2	23	
Pen 15:					
White Leghorn, fryer flesh...	25	164	-5.8	24	Polyneuritis. Died. Polyneuritis. Do.
Autoclaved rice.....	75	165	-26.7	50	
		166	-21.3	29	
		167	-12.0	20	
Average.....			-16.5	31	
Pen 16:					
White Leghorn, fryer flesh...	30	168	-15.9	25	Polyneuritis. Died. Polyneuritis. Do.
Autoclaved rice.....	70	169	-18.7	45	
		170	-8.6	22	
		171	-24.1	42	
Average.....			-16.8	34	

a Bird's crop congested, did not weigh.

ANTINEURITIC VITAMIN IN THE FLESH OF HENS

In Table III are shown the results of feeding tests with the dried flesh of Rhode Island Red, Barred Plymouth Rock, and White Leghorn hens as the source of vitamin B in the rations. The percentages of dried flesh in the several rations ranged from 15 to 30. The results of these experiments show that the flesh of each of the three lots of hens had a very low antineuritic value. The pigeons in pen 20 which were fed a ration containing 15 per cent of Rhode Island Red hen flesh show the maximum survival period, 28 days, while those in pen 18 which were fed a ration containing 20 per cent of Barred Plymouth Rock hen flesh show the minimum average survival period of 19 days. Not a pigeon survived the test period of 56 days, the maximum survival period for an individual bird being 37 days. All pigeons receiving the hen-flesh rations lost in weight, the individual losses ranging from 3.7 to 41.4 per cent, and the average pen losses from 15.1 to 28.1 per cent.

On the whole, it appears that the flesh of the hens had an appreciably lower content of the antineuritic vitamin than did that of the frying chickens. This difference is probably due to the fact that the young chickens were on free range and received a greater abundance of green feed than the hens which were confined in yards, although the latter received a certain amount of green feed.

TABLE III.—*Antineuritic value of hens' flesh*

Ration		Pigeon No.	Change in weight	Survival period	Result
Ingredients	Per cent				
Pen 17:			<i>Per cent</i>	<i>Days</i>	
Barred Plymouth Rock, hen flesh.	15	109	-24.5	17	Polyneuritis.
Autoclaved rice.....	85	110	-18.4	16	Do.
		111	-41.4	26	Died.
		112	-27.7	28	Polyneuritis.
Average.....			-28.0	22	
Pen 18:					
Barred Plymouth Rock, hen flesh.	20	113			Discarded, poor condition.
Autoclaved rice.....	80	114	-11.6	16	Polyneuritis.
		115	-31.7	21	Do.
		116	-26.5	21	Do.
Average.....			-23.3	19	
Pen 19:					
Barred Plymouth Rock, hen flesh.	30	213	-28.6	35	Polyneuritis.
Autoclaved rice.....	70	214	-24.1	37	Do.
		215	-18.6	18	Do.
		216	-7.6	17	Do.
Average.....			-19.7	27	
Pen 20:					
Rhode Island Red, hen flesh.	15	117			Accidental death.
Autoclaved rice.....	85	118	-23.4	24	Polyneuritis.
		119	-17.9	23	Do.
		120	-31.6	37	Do.
Average.....			-24.3	28	
Pen 21:					
Rhode Island Red, hen flesh.	20	121	-18.2	16	Polyneuritis.
Autoclaved rice.....	80	122	-9.7	18	Do.
		123	-33.4	33	Do.
		124	-33.0	23	Do.
Average.....			-23.6	23	
Pen 22:					
Rhode Island Red, hen flesh.	25	201	-16.0	22	Polyneuritis.
Autoclaved rice.....	75	202	-25.5	21	Do.
		203	-18.1	20	Do.
		204	-19.2	26	Do.
Average.....			-19.7	22	
Pen 23:					
Rhode Island Red, hen flesh.	30	205			Poor condition, discarded.
Autoclaved rice.....	70	206	-22.5	28	Polyneuritis.
		207	-21.7	25	Do.
		208	-16.4	17	Do.
Average.....			-20.2	23	
Pen 24:					
White Leghorn, hen flesh.....	15	125	-14.2	27	Polyneuritis.
Autoclaved rice.....	85	126	-26.4	35	Do.
		127	-25.6	24	Do.
		128	-7.9	23	Do.
Average.....			-18.5	27	
Pen 25:					
White Leghorn, hen flesh.....	20	129	-25.0	32	Polyneuritis.
Autoclaved rice.....	80	130	-3.7	16	Do.
		131	-31.8	19	Do.
		132			Escaped.
Average.....			-20.2	22	
Pen 26:					
White Leghorn, hen flesh.....	25	217	-37.1	35	Polyneuritis.
Autoclaved rice.....	75	218	-29.3	24	Do.
		219			Died from injury.
		220	-18.0	21	Polyneuritis.
Average.....			-28.1	27	
Pen 27:					
White Leghorn, hen flesh.....	30	221	-17.1	24	Polyneuritis.
Autoclaved rice.....	70	222	-20.4	24	Do.
		223	-12.1	26	Do.
		224	-10.7	24	Do.
Average.....			-15.1	25	

ANTINEURITIC VITAMIN IN FLESH OF TURKEYS, DUCKS, AND GUINEA FOWL

The results of the feeding tests with the dried flesh of turkeys, ducks, and guinea fowl are reported in Table IV. Each kind of flesh was fed only to one pen of pigeons, 30 per cent of the flesh being used in each ration. The data presented in this table show that the turkey flesh had a rather low antineuritic value, approximately the same as that of the frying chickens reported in Table II. The average survival period with the turkey flesh was 35 days and the loss in weight 23.7 per cent. All the pigeons receiving the ration containing 30 per cent of turkey flesh developed polyneuritis during the test period of 56 days. The guinea-fowl flesh had a somewhat higher antineuritic value than the turkey flesh, the average survival period of the pen of pigeons receiving a ration containing 30 per cent of the guinea-fowl flesh being 44 days, the maximum survival period 49 days, and the average loss in weight 22 per cent. The duck flesh had the highest antineuritic value of any of the lots of poultry flesh tested. The ration containing 30 per cent of dried duck flesh fully protected 4 pigeons against polyneuritis during the test period of 56 days. Two pigeons lost 6.3 and 10.7 per cent in weight, but the other two gained 3.3 and 9.8 per cent, the average change being a loss of 1 per cent.

TABLE IV.—Antineuritic value of turkey, duck, and guinea-fowl flesh

Ration		Pigeon No.	Change in weight	Survival period	Result
Ingredients	Per cent				
Pen 28:			<i>Per cent</i>	<i>Days</i>	
Turkey flesh.....	30	237	-16.7	32	Polyneuritis. Do. Do. Do.
Autoclaved rice.....	70	238	-19.3	28	
		239	-34.5	41	
		240	-24.2	39	
Average.....			-23.7	35	
Pen 29:					
Duck flesh.....	30	241	-10.7	56	Fair condition at end of test. Good condition at end of test. Do. Fair condition at end of test.
Autoclaved rice.....	70	242	+9.8	56	
		243	+3.3	56	
		244	-6.3	56	
Average.....			-1.0	56	
Pen 30:					
Guinea fowl flesh.....	30	233	-16.9	39	Polyneuritis. Do. Do. Do.
Autoclaved rice.....	70	234	-20.9	49	
		235	-29.0	41	
		236	-21.0	48	
Average.....			-22.0	44	

ANTINEURITIC VITAMIN IN HENS' GIZZARDS AND LIVERS

In Table V are reported the antineuritic values obtained for the dried gizzards and livers from hens. The data presented show that the gizzards had a very low antineuritic value, the average survival period of the two pens of birds receiving rations containing 15 and 20 per cent of dried gizzards being only 21 and 22 days, respectively. The hens' livers had a fairly high antineuritic value, the average survival period of the pen of pigeons receiving the ration containing 15 per cent of dried

liver being 38 days, and the average loss in weight only 4.4 per cent. One bird survived the test period of 56 days, having gained 12.5 per cent in weight, while the three others lost in weight and developed polyneuritis.

The ration which contained 20 per cent of dried hens' liver fully protected a pen of 3 pigeons against polyneuritis and loss in weight for 56 days. Only 3 pigeons were used in this pen on account of the limited supply of hens' livers.

TABLE V.—*Antineuritic value of hens' gizzards and livers.*

Ration		Pigeon No.	Change in weight	Survival period	Result
Ingredients	Per cent				
Pen 31:			<i>Per cent</i>	<i>Days</i>	
Hens' gizzards .....	15	133	—12.3	27	Polyneuritis.
		134	—24.7	24	Do.
Autoclaved rice .....	85	135	—8.8	17	Do.
		136	—25.5	16	Do.
Average .....			—17.8	21	
Pen 32:					
Hens' gizzards .....	20	137	—12.9	25	Polyneuritis
		138	—13.8	20	Do.
Autoclaved rice .....	80	139	—9.7	20	Do.
		140			Escaped.
Average .....			—12.1	22	
Pen 33:					
Hens' liver .....	15	141	+12.5	56	Good condition at end of test.
		142	—5.6	32	Polyneuritis.
Autoclaved rice .....	85	143	—16.7	44	Do.
		144	—7.7	19	Do.
Average .....			—4.4	38	
Pen 34:					
Hens' liver .....	20	145	0.0	56	Good condition at end of test
		146	+4.8	56	Do.
Autoclaved rice .....	80	147	+4.1	56	Do.
Average .....			+3.0	56	

## ANTINEURITIC VITAMIN IN HENS' EGGS

In Table VI are reported the antineuritic values obtained for White Leghorn, Barred Plymouth Rock, and Rhode Island Red eggs. Each lot of dried eggs was fed to 4 pens of 4 pigeons each. The results of these experiments show that the eggs from each of the three breeds of chickens had a rather low antineuritic value. The average survival periods of the pigeons getting rations containing 25 and 30 per cent, respectively, of the eggs from each breed of chickens were as follows: White Leghorn, 33 days; Barred Plymouth Rock, 31 days, and Rhode Island Red, 27 days, while the average losses in weight were 11.5, 8.5, and 7.3 per cent, respectively. The rations containing the smaller percentages of eggs had about the same antineuritic values as the check rations in Table I, while the rations containing as much as 25 or 30 per cent of eggs had slightly higher values. Only 2 pigeons out of the 48 that were fed the egg rations survived the test period of 56 days; one was in fair condition at the close, while the other was greatly emaciated. It is to be noted, however, that while the average survival period of the pigeons receiving rations containing 30 per cent of dried eggs are not high, yet the average losses in weight are relatively low.



TABLE VI.—*Antineuritic value of raw hens' eggs*

Ration		Pigeon No.	Change in weight	Survival period	Result
Ingredients	Per cent				
Pen 35:			<i>Per cent</i>	<i>Days</i>	
White Leghorn eggs.....	5	1, 201	-32.9	25	Polyneuritis.
Autoclaved rice.....	95	1, 202	-27.2	17	Do.
		1, 205	-28.8	29	Do.
		1, 206	-28.0	24	Do.
Average.....			-29.2	24	
Pen 36:					
White Leghorn eggs.....	10	1, 208	-30.0	29	Polyneuritis.
Autoclaved rice.....	90	1, 209	-23.9	25	Do.
		1, 210	+6.1	21	Do.
		1, 211	-17.8	24	Do.
Average.....			-16.4	25	
Pen 37:					
White Leghorn eggs.....	25	172	-4.1	20	Polyneuritis
Autoclaved rice.....	75	173	-25.3	29	Do.
		174	-19.5	33	Do.
		175	-5.5	27	Do.
Average.....			-13.6	27	
Pen 38:					
White Leghorn eggs.....	30	176	-2.5	56	Fair condition at end of test.
Autoclaved rice.....	70	177	-15.4	24	Polyneuritis.
		178	-10.3	36	Do.
		179	-----	a 11	Died, cause unknown.
Average.....			-9.4	39	
Pen 39:					
Barred Plymouth Rock eggs.....	5	1, 213	-20.8	29	Polyneuritis.
		1, 214	-37.0	35	Extreme emaciation, discontinued.
Autoclaved rice.....	95	1, 215	-9.5	18	Polyneuritis.
		1, 216	-25.4	24	Do.
Average.....			-23.2	27	
Pen 40:					
Barred Plymouth Rock eggs.....	10	1, 217	-2.1	24	Polyneuritis.
Autoclaved rice.....	90	1, 218	+2.6	17	Do.
		1, 219	+2.3	17	Do.
		1, 220	-22.2	31	Do.
Average.....			-4.9	22	
Pen 41:					
Barred Plymouth Rock eggs.....	25	180	-10.4	29	Polyneuritis.
Autoclaved rice.....	75	181	-8.4	49	Do.
		182	-19.2	35	Do.
		183	-15.7	17	Do.
Average.....			-13.4	33	
Pen 42:					
Barred Plymouth Rock eggs.....	30	184	-6.0	24	Polyneuritis.
Autoclaved rice.....	70	185	-2.6	26	Do.
		186	-7.6	45	Do.
		187	+2.0	22	Do.
Average.....			-3.6	29	
Pen 43:					
Rhode Island Red eggs.....	15	101	-16.9	30	Polyneuritis.
Autoclaved rice.....	85	102	-12.3	20	Do.
		103	-13.0	24	Do.
		104	-21.8	37	Do.
Average.....			-16.0	28	
Pen 44:					
Rhode Island Red eggs.....	20	105	+1.1	30	Polyneuritis.
Autoclaved rice.....	80	106	-13.2	7	Do.
		107	-9.8	37	Do.
		108	-15.8	48	Do.
Average.....			-9.4	31	
Pen 45:					
Rhode Island Red eggs.....	25	188	-13.3	29	Polyneuritis.
Autoclaved rice.....	75	189	-35.4	56	Emaciated at end of test.
		190	+1.3	21	Polyneuritis.
		191	-2.2	15	Do.
Average.....			-12.7	30	
Pen 46:					
Rhode Island Red eggs.....	30	192	-5.2	25	Polyneuritis.
Autoclaved rice.....	70	193	-1.7	24	Do.
		194	-5.1	17	Do.
		195	+0.6	30	Do.
Average.....			-2.9	24	

a Not included in average.

EFFECT OF COOKING UPON ANTINEURITIC VITAMIN IN EGGS

White Leghorn eggs were cooked in boiling water for approximately 7 minutes and then cooled in a stream of cold water. The contents of the eggs were separated from the shells, ground in a meat grinder, dried in a current of air at a temperature below 60° C., and then ground in a burr mill. The dried eggs were mixed with autoclaved rice in two proportions and the antineuritic values of the rations are reported in Table VII.

By referring to Table VI, pen 38, it will be noted that the average survival period of that pen of birds receiving a ration containing 30 per cent of dried raw White Leghorn eggs is 39 days, while the average survival period of the pigeons in pen 48 that were fed a ration containing 30 per cent of dried cooked White Leghorn eggs is practically the same, or 40 days. The pigeons receiving 30 per cent of the raw eggs lost an average of only 9.4 per cent in weight, while those that were fed the same proportion of cooked egg lost an average of 19.2 per cent. It appears, then, that the antineuritic value of the eggs was slightly reduced by the method of cooking employed.

TABLE VII.—Antineuritic value of dried cooked hens' eggs.

Ration		Pigeon No.	Change in weight	Survival period	Results
Ingredients	Per cent				
Pen 47:			<i>Per cent</i>	<i>Days</i>	
Cooked White Leghorn eggs.	15	225	—24.4	22	Polyneuritis. Do. Died. Polyneuritis
Autoclaved rice.....	85	226	—17.3	19	
		227	—29.7	46	
		228	—14.1	15	
Average.....			—21.4	26	
Pen 48:					
Cooked White Leghorn eggs.	30	229	—15.6	29	Polyneuritis. Emaciated at end of test. Polyneuritis. Do.
Autoclaved rice.....	70	230	—34.8	56	
		231	—10.3	29	
		232	—15.9	46	
Average.....			—19.2	40	

DISCUSSION OF RESULTS

POULTRY FLESH

The relatively low antineuritic value that was found for the flesh of the frying chickens is surprising in view of the fact that the birds were grown on free range with an abundant supply of green feed, and in addition were fed mixed grains and mash that undoubtedly contained an ample supply of vitamin B.

The flesh of the hens was even more deficient in vitamin B, although they also were fed grain and mash that contained an ample supply of the vitamin, and in addition received considerable green feed, but not nearly so much as the young chickens. The slightly higher antineuritic value of the young chickens is probably due to the greater abundance of green feed in their diet. Apparently neither the young chickens nor the hens had the ability to store any considerable quantity of vitamin B in their muscle tissue.

There seem to have been no significant differences in the vitamin B content of the flesh of the three breeds of chickens. This applies both to the frying chickens and to the hens.

The turkey flesh had a rather low antineuritic value, similar to that of the frying chickens, while the flesh of the guinea fowl had a somewhat higher value. The flesh of the ducks had a considerably higher antineuritic value than that of any of the other fowl tested.

Since no information is available regarding the feeding of the turkeys, ducks, and guinea fowl, it can not be stated whether the higher antineuritic value of the duck and guinea-fowl flesh, as compared with that from the chickens and turkeys, may have been due to a difference in the vitamin B content of the rations of the birds or not. It is very unlikely, however, that the diet either of the ducks or the

guinea fowl was any richer in vitamin B than was that of the frying chickens. The facts available suggest that perhaps the ducks and the guinea fowl had greater capacity to store vitamin B in their muscle tissue than had the chickens. More evidence is necessary, however, before a positive conclusion can be drawn.

The hens' gizzards had a very low antineuritic value, but the hens' livers had a fairly high value, higher than that of any of the samples of poultry flesh. Twenty per cent of the dried hens' liver in a ration with autoclaved rice fully protected a pen of 3 pigeons against both polyneuritis and loss in weight during a period of 56 days. The hens' liver had a somewhat lower antineuritic value, however, than the liver of the ox, sheep, or hog, as previously reported by one of the writers (2).

#### EGGS

The results of our experiments show no significant differences in the vitamin B content of the eggs from the three breeds of chickens. The rather low antineuritic values that were obtained are somewhat surprising in view of the statements in the literature concerning the vitamin B content of eggs (1, 4, p. 12, 6, p. 86). A careful perusal of the article by Cooper (1) indicates, however, that his experimental data, if judged by the standards employed by the writers, do not show a high antineuritic value for eggs, but, rather, a relatively low one. For example, Cooper found that 10 grams of raw, undried egg yolk daily in the ration of each of 2 pigeons protected them against polyneuritis for 55 days, but that they had lost 3 and 7.5 per cent in weight, respectively. This quantity of egg yolk corresponds to 7 grams of dried whole egg, and would amount to 46.6 per cent of the ration fed daily to a 300-gram pigeon in our experiments.

#### SUMMARY

The investigations reported in this paper are not sufficiently comprehensive to warrant any final conclusion as to the vitamin B content of poultry flesh and eggs as found on the markets in this country, and the results are therefore offered simply as a contribution to our knowledge of the subject. It is realized that additional information concerning the vitamin B content of this class of products is much to be desired.

The results of the vitamin B studies with poultry flesh and eggs may be summarized as follows:

1. The flesh from the hens was relatively deficient in the antineuritic vitamin, while the flesh from the growing chickens had a slightly higher value.
2. The turkey flesh had a rather low antineuritic value.
3. The flesh from guinea fowl contained a fair supply of the antineuritic vitamin.
4. The duck flesh was richer in the antineuritic vitamin than that from any of the other fowls.
5. The hens' livers had the highest antineuritic value of any of the products tested, while the gizzards had a very low value.
6. The hens' eggs tested had a rather low antineuritic value.

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# DISSEMINATION OF THE STEM AND BULB INFESTING NEMATODE, *TYLENCHUS DIPSACI*, IN THE SEEDS OF CERTAIN COMPOSITES<sup>1</sup>

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The investigations recounted in this paper were carried on as a result of the finding of the widespread occurrence of the stem-nematode-infested false dandelion, *Hypochaeris radicata*, in the Pacific Coast States, as reported by Godfrey and McKay (2).<sup>2</sup> My subsequent discovery of the same nematode in the true dandelion, *Taraxacum officinale*, over a wide area in northeastern United States in July and September, 1923, made the problem of the manner of dissemination the more interesting.

## REVIEW OF LITERATURE

A study of the literature has shown that both of these hosts are more or less abundantly infested by nematodes in Europe,<sup>3</sup> as indicated by their frequent mention by writers on plant galls. In 1885 Trail (19, p. 211) listed *Hypochaeris radicata* as subject to a gall caused by *Tylenchus* sp. He reported observing it first in Scotland, in 1882. This seems to be the earliest authentic report. In 1886 Liebel (6, p. 547, No. 124) listed it as subject to a "Helminthoecidium" which he described thus:

Blattparenchym gallen: grünlichgelbe längliche Auftreibung der Blattmittellrippe, seltener ründliche bis längliche Auftreibung der Spreite. Die Aelchen darin in Anzahl.

Houard (3, p. 1035, No. 6040) in a comprehensive list of plant galls of Europe in 1909, listed this plant as having galls produced by "Anguillulide," Massalongo (9, p. 8, No. 7) in 1915 listed it as subject to a "Helminthoecidium" in Italy, described the symptoms, and illustrated with drawings typical affected leaves and stems, identical in appearance with the disease produced in this country by *Tylenchus dipsaci* (Kühn) Bast. Thus this plant is reported all the way from Scotland to Italy as subject to galls produced by nematodes, it seems safe to assume by *T. dipsaci*.

The true dandelion, *Taraxacum officinale*, was mentioned by Thomas in 1885 (15) as subject to a "Helminthoecidium," and more completely described the next year (16, p. 304, No. 49). The causal organism was referred to as *Tylenchus* sp. in a still later paper (17). It was found, in this instance, in "Grösster Menge auf den Wiesen um Cogne, Gimilian und Lillian, Piemont, bei 1,500 to 1,800 m. Meereshöhe." Liebel (6, p. 573, No. 288), in 1886, reported a "Helminthoecidium" on this host at Bolchen, Lorraine. Rübsaamen reported it in 1890 (13, p. 53, No. 207) in "Die Gallmücken und Galle des Siegerlandes." Houard (3, p. 1042, No. 6037) lists it for northern, central, and southern Europe as a *Tylenchus* gall. With *Taraxacum* again, though the specific parasite is not named, *Tylenchus dipsaci* is strongly indicated in every case.

<sup>1</sup> Received for publication Mar. 20, 1924.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 478.

<sup>3</sup> I gratefully acknowledge my indebtedness to Dr. G. Steiner, Office of Crop Technology, Bureau of Plant Industry, for his help in finding the pertinent European literature.

European writers report various other composites as subject to nematode galls, most of which, judging from the descriptions given (except the root galls) are probably due to *Tylenchus dipsaci*. These are tabulated herewith:

TABLE I.—Composite plants subject to Nematode galls

Host plant	Nematode as listed	Date	Authority
<i>Antennaria dioica</i> (L.) Gaertn. ....	Anguillulidæ .....	1909	Houard (5).
<i>Atractylis gummifera</i> L. ....	do .....	1912	Do.
<i>Carduus defloratus</i> L. ....	Tylenchus sp. ....	1896	Thomas (18).
<i>Carlina corymbosa</i> L. ....	do .....	1909	Houard (5).
<i>Cichorium intybus</i> L. ....	Anguillulidæ .....	1902	Geisenheyner (1).
<i>Cirsium oleraceum</i> (L.) Scop. ....	do .....	1896	Thomas (18).
<i>Cirsium arvense</i> (L.) Scop. ....	do .....	1902	Geisenheyner (1).
<i>Cirsium bulbosum</i> (Lam.) DC. ....	do .....	1902	Do.
<i>Crepis foetida</i> L. ....	Tylenchus devastatrix .....	1912	Houard (5).
<i>Crepis leontodontodes</i> All. ....	Tylenchus sp. ....	1905	Trotter (20).
<i>Crepis taraxacifolia</i> Thuill. ....	Tylenchus devastatrix .....	1906	Maige (8).
<i>Cynara cardunculus</i> L. ....	Tylenchus dipsaci .....	1912	Stefani Perez (14).
<i>Hieracium auricula</i> L. ....	do .....	1903	Lagerheim (see Houard (5) ).
<i>Hieracium pilosella</i> L. ....	do .....	1883	Trail (19).
		1885	Liebel (6) and several others.
<i>Leontodon hastilis</i> L. ....	Anguillulidæ .....	1885	Löw Fr. (7).
<i>Leontodon hispidus</i> L. ....	Tylenchus sp. ....	1907	Mariani (see Houard (5) ).
		1896	Do.
<i>Leontodon incanus</i> Schrank .....	Anguillulidæ .....	1901	Kieffer (4).
<i>Leontodon pyrenaicus</i> Gouan. ....	Tylenchus sp. ....	1885	Löw, F. (7).
		1903	Lagerheim (see Houard (5) ).

In only one of these cases, that of *Crepis taraxacifolia*, reported by Maige (8), was mention made of transmission of the nematode by seed. He described a condition of the flower head (capitule) very similar to that which occurs with the composites reported upon in this paper. He illustrated this condition with drawings, and treated of dissemination by the seed as follows:

Les capitules attaqués portant des fruits d'apparence normale, bien que remplis de larves enkystées, ces fruits peuvent grâce a leurs aigrettes, être transportés par le vent à de grandes distances et constituent ainsi pour le *Tylenchus devastatrix* un moyen de propagation des plus favorables.

The original paper on the stem nematode by Kühn (5) dealt with the organism in *Dipsacus fullonum* in the Dipsaceae, a family closely allied to the Compositae. Kühn wrote in this connection:

Diese Krankheit ist characterisirt durch ein allmähiges Missfarbigwerden und Vertrocknen der Blütenköpfe; Der Pappus des gesunden Samens ist gestielt, bei den kranken Körnern ist er fast doppelt so gross und sitzend. Die kranken Körner sind nicht vollständig mit Auguillulen ausgefüllt, vielmehr findet sich in denselben noch der verkümmerte Samenkern, während die ersteren zu weisslichen Häufchen vereinigt in dem Gewebe der abnorm verdickten Samenschale, namentlich am Grunde derselben vorhanden sind.

Thus he established the presence of the nematodes within the seed head and seed of *Dipsacus fullonum*.

## DISTRIBUTION OF NEMATODE-INFESTED COMPOSITES IN AMERICA

### HYPOCHAERIS RADICATA

As stated in the paper by Godfrey and McKay (2), the stem nematode occurs along the west coast of America all the way from Tacoma, Wash., to San Francisco, Calif., in *Hypochaeris radicata*. This plant is reported to have been introduced into that region. In this regard Piper and Beattie (11) stated in their Flora of the Northwest Coast: "A very troublesome weed in lawns and pastures; introduced from Europe." Nelson (10) listed it as a possible introduction with ballast dirt. Mr. M. W. Gorman, botanist, now curator of the Portland Historical Society, at the Forestry Building, Portland, Oreg., who is also familiar

with ballast flora, considers this a very great probability. He is familiar with the early appearance of the plant in the Northwest, and with its very rapid spread and increase until now it is one of the most common and troublesome of lawn and meadow weeds. He referred me to Fort Nisqually, near Olympia, Wash., as the site of an early ballast dump, and the very possible point of introduction of the weed. True enough, in 1923 I found *Hypochaeris* in that vicinity in unusually great abundance as a roadside, lawn, and garden weed. Early settlers in that region stated to me that they knew when it first appeared, approximately 50 years ago.

The interesting observation was made as well, that the parasitic nematode *Tylenchus dipsaci* was observed to be very abundant in that region, possibly even more so than where first observed in western Oregon, and certainly more abundant than in its southern range, in western California. It would appear to be very plausible to suppose that the parasite was introduced along with the host plant, and that the parasite as well as the host plant have become simultaneously widespread.

#### CREPIS VIRENS

While studying the flora associated with diseased *Hypochaeris*, I found a few typically affected plants of *Crepis virens*, first at Eureka, Calif., and later at Seaside, Oreg. They were by no means as abundant as affected *Hypochaeris radicata*, and merit attention only in the fact that they constitute a new host record for the organism.

#### TARAXACUM OFFICINALE

Stem-nematode-infested dandelions, *Taraxacum officinale*, were first encountered at Williamson, in Wayne County, N. Y., in July, 1923. Subsequently they were found occasionally all the way between Ithaca and Niagara Falls, and across the Niagara River, in the Province of Ontario, Canada. Plant disease survey forces in the State eventually reported the disease to be present in 14 counties in western New York. In September I found it among dandelions growing in a low meadow in Arnold Arboretum, in Boston, Mass.

With the true dandelion, therefore, as with the false dandelion in the West, considerable distribution of the parasite has taken place.

#### RELATION OF THE NEMATODE TO THE SEED

When first observations were made on nematode-diseased *Hypochaeris*, the plant was not flowering, consequently the disease was to be found only in the leaves. (2, Pl. 2). Later summer observations, however, disclosed the presence of swellings in the flower pedicels. The abundant occurrence of stem swellings due to an insect, *Aulax hypochaeridis* (Pl. 1, A) were at first confusing. Characteristic nematode swellings became evident, however, usually in the pedicel at the base of the flower head. These were irregular, resulting sometimes in considerable distortion, and were distinctly soft, rather than hard as were the insect galls. Typical blossoms in this condition, compared with the normal, are shown in Plate 1, B and C. As stated previously, the swellings pictured by Massalongo (9) are identical in appearance with these.

Such affected stems of *Hypochaeris* when cut longitudinally showed brownish discoloration in the receptacle, and for a short distance below, in the pith of the stem. This condition is illustrated in Plate 1, D. The discolored tissue was found to contain an abundance of living, active *Tylenchus* in all stages of development.

Flowers in various stages of development were studied. By microscopic examination it was seen that some of the very young seed were discolored at their

point of attachment with the receptacle. These were found to contain nematodes. The eelworms could readily be seen with the binocular coiled up in the interior, through the semitransparent achene wall, as shown by Plate 2, A. Seeds in more advanced stages of development were found, upon dissection, to contain living nemas. One showed a nema extending from the base. Another (Pl. 2, B) showed one at the upper extremity, at the base of the pappus stalk. Some of the seeds were killed by the invasion, as shown by their completely collapsed condition. In some of the invaded seeds, however, such injury did not occur, for the seed was intact, since the eelworms were present only between the achene wall and the testa of the seed.

Experimental plantings of seed from diseased flower heads produced a reduced stand of seedlings, among which were several typically diseased plants. Swellings appeared first in the petioles of the primary leaves, and subsequently in one or two of the permanent leaves. Plate 2, C, shows one of the affected seedlings with characteristic swelling in the midrib of the first secondary leaf. Examination of such a plant three days after infection was first evident disclosed larvae of *Tylenchus dipsaci* not yet quite sexually mature. In all invaded seed observed, the pappus was apparently normal and the seed capable of being transported by the wind. Plate 2, D, shows several mature seed of *Hypochaeris radicata*. Some of this same lot were later shown to contain living nemas.

In the true dandelion (*Taraxacum officinale*) exactly the same conditions existed. The leaf symptoms are identical with those previously described for *Hypochaeris radicata*. Plate 3, C, shows typical leaf swellings. Plate 3, A, shows one of many nematode-infested heads found, this one sectioned to show the discolored receptacle and seed bases. More than 50 per cent of the seed in this particular head were invaded by nemas. One of the seeds with its pappus is pictured in Plate 3, B. The seed is undamaged in spite of the fact that a nematode was present beneath the outer seed coat.

A study of growing plants made it evident how primary invasion of the seed head takes place. Both *Taraxacum* and *Hypochaeris* grow as a "rosette" on the ground, with flat leaves radiating from a common center. The outer ends of the leaves often are higher than the inner, thus permitting dew and rain to flow in toward the center, carrying with them free nemas that have migrated out of infested spots in the leaves. *Tylenchus dipsaci* was thus observed many times in the young, actively growing central region of the plants, some of them free from the host tissues, others within the young, highly susceptible parts. Without doubt much of the leaf infection takes place when the leaves are young. It is likewise in this region that the flower heads form and develop. Ready infestation of the flower head can thus take place, and, indeed, many young heads were observed that were thus infested. Plate 3, D, a photograph of a dandelion plant sectioned longitudinally, shows two flower heads in different stages of development, and their obviously favorable location, when young, for infection by nemas. The well-known rapid growth of the pedicel after the flower is fully formed is a factor which favors the dissemination of the invading parasites, as well as of the seed, by the agency of the wind.

It is evident that dissemination of the nemas by the seed is easily accomplished, and under natural conditions it undoubtedly takes place to a large extent. If the seed has been killed, the eelworms released may still infest plants that are already established. If the infested seed is alive then a new colony is established at once, with the host plant immediately available. Thus is explained the distribution of the nematode parasite to an extent that is practically coequal in range with that of its host, the false dandelion, in the Pacific Coast States, and the probably widening circle of infestation in the true dandelion in the Northeast.

## SEEDS OF OTHER PLANTS LIKEWISE NEMATODE CARRIERS

The dissemination of parasitic stem nematodes undoubtedly takes place by means of the seed of other composites and allied plants in a similar manner. Reference has already been made to the observations of Maige (8) in *Crepis taraxacifolia*. Geisenheyner (1) described a gall due to "Anguillulides" in *Cirsium bulbosum* in which the swelling is situated at the base of the flower head. (3, No. 5915, p. 1016), as it is in *Hypochaeris* and *Taraxacum* in America. A like condition exists with *Leontodon incanus*, in which the "Tige est epaissie et tordue, au-dessous de l'inflorescence, sur un longueur de 20 mm. et plus," etc., according to Houard (3, No. 6048, p. 1036). Likewise, in *Hieracium pilosella* "Capitule gonflé et contourné demeurant fermé, porté sur une hampe florale renflée." (Houard, 3, No. 1698, p. 1057). In all probability the seeds of all these plants are penetrated by the nemas, without injuring their capacity of being carried by the wind.

In *Dipsacus fullonum*, a member of the family Dipsaceae, which is very close to the Compositae, the same manner of dissemination undoubtedly occurs, as already cited (5). Ritzema Bos (12) mentioned infestation in onion seedlings (*Allium cepa*) in the earliest stages of their development. A large part of this was due to the fact that penetration had taken place from infested soil shortly after the appearance of the cotyledon through the seed coat. In addition, however, he found *Tylenchus dipsaci* in the blossoms of mature plants, and in one case 3 per cent of the seeds were infested. In many other plants whose seeds are not windblown it is possible that the nemas enter the seed and are carried with them.

Dissemination of the plant parasitic nematodes on the surface of seed undoubtedly takes place to a large extent as well. This will be treated at length in another paper in connection with experimental work on the clover and alfalfa stem nematode.

## SIGNIFICANCE OF WIND DISSEMINATION OF THE STEM NEMATODE

It is too early to make any estimate as to whether or not this great spread of the stem nematode in the composites may be of any economic significance. Indeed, any disease that even suggests the possible lessening of the spread of either the false or the true dandelion might be looked upon as a blessing rather than otherwise, except possibly in connection with the few fields of cultivated *Taraxacum* grown for food. Thus far the indications are that the nemas infesting the composites are specialized physiological races not capable of infesting others of the known economic host plants. There are indications of gradual adaptation to new host plants, however. Further investigations are being made in this connection.

## SUMMARY

(1) The leaf and stem infesting nematode, *Tylenchus dipsaci* Kühn, has been found to be abundant on the false dandelion, *Hypochaeris radicata*, along the Pacific coast, from Tacoma, Wash., to San Francisco, Calif. It occurs as well in the true dandelion, *Taraxacum officinale*, in Western New York, the Province of Ontario, Canada, and at Boston, Mass. According to the literature, it occurs abundantly on these and other composites in Europe.

(2) In addition to producing swellings and distortions of the leaves, the nemas penetrate the developing flower head and produce more or less distortion in that region. Furthermore, in the case of the false and true dandelion they were observed to have actually penetrated into the interior of the seed.

(3) This accounts definitely for their wide distribution on these hosts, for the wind carries the nematode-infested equally well with the nematode-free seed.



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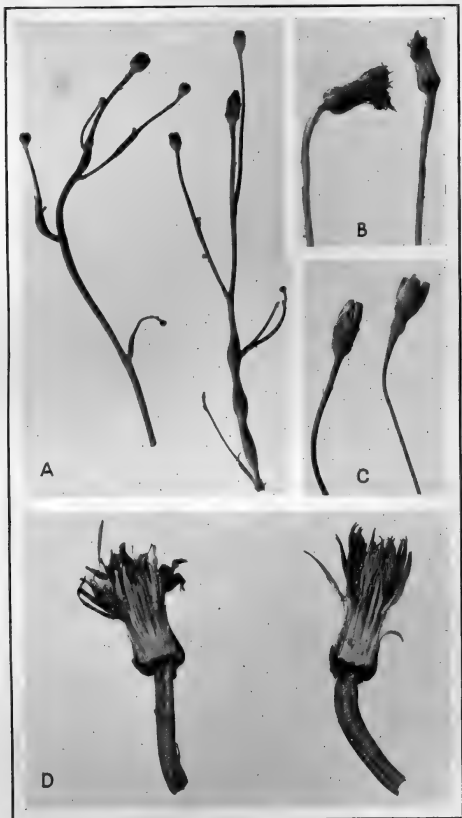
## PLATE 1

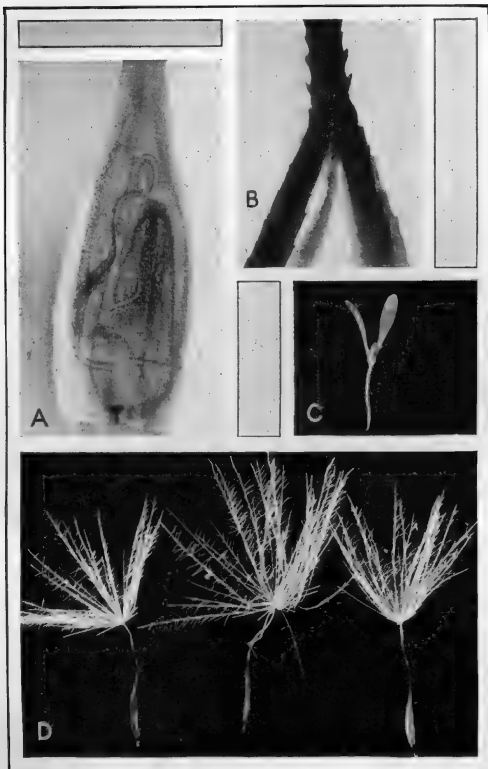
A.—Stems of false dandelion, *Hypochaeris radicata*, showing galls produced by the insect *Aulax hypochaeridis* Kieff. These are shown to distinguish them from the galls caused by the stem nematode, *Tylenchus dipsaci*.

B.—Immature flower heads of false dandelion showing stem nematode swellings at the base of the flower head.

C.—Normal flower heads of *H. radicata*; compare with B.

D.—Sections through nematode-infested heads of *H. radicata*. Note the discolored receptacle, which contains hundreds of nematodes.





## PLATE 2

A.—Photograph of immature seed of *Hypochaeris radicata*, showing nemas coiled inside the seed; taken by transmitted light.

B.—Apical end of a mature seed, showing nematodes within the seed.

C.—Small seedling of *Hypochaeris radicata* showing typical *Tylenchus* swelling in the midrib of the first permanent leaf. This is one of several such plants that resulted from planting seed from a mature infested flower head into a flat of sterilized soil in the greenhouse.

D.—Three typical seed of *Hypochaeris radicata*, taken from an infested flower head. About half the seed from this same lot contained living nematodes.

### PLATE 3

A.—A flower head of true dandelion, *Taraxacum officinale*, broken open longitudinally to show the slightly discolored receptacle and seed bases. More than 50 per cent of these seeds contained living *Tylenchus dipsaci*.

B.—A single seed of *T. officinale*, from which two living Tylenchi were dissected. Note the seed proper, which was intact, and the normal pappus.

C.—A young plant of *Taraxacum officinale* with three leaves showing typical *Tylenchus* deformities.

D.—A dandelion plant sectioned longitudinally through the crown to show the developing flower heads. Note that when very small they are in the very heart of the plant, protected from ordinary mechanical injury, unquestionably, but ideally situated for infection by the stem nematode.



# SOME MODIFICATIONS OF THE PICRIC ACID METHOD FOR SUGARS<sup>1</sup>

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## INTRODUCTION

The writers recently had occasion to use the picric acid colorimetric method (commonly called the Benedict-Lewis method)<sup>2</sup> for determining the sugars in cornstalk juices.<sup>3</sup> Although it has been used mostly as a micro method for blood and urine analyses, it has been used to some extent as a macro method. (3,<sup>4</sup> 5, 12.) It proved to be more rapid than, and approximately as accurate as, a copper reduction method after certain modifications had been introduced to eliminate certain sources of error. The following is a description of the errors met with and the corresponding modifications.

## METHOD

The basic procedure, the various items of which were made the subject of the present investigation, was taken from Rose (16) and is as follows:

One cubic centimeter of sugar solution of proper concentration is transferred to a long narrow test tube (so-called sugar tube, graduated to 10 cc.) containing 2 cc. of saturated picric acid and 1 cc. of 20 per cent sodium carbonate solution. The test tube is then immersed in boiling water for 20 to 30 minutes to effect reduction of the picrate to picramate. The solution is then made up to volume. The color present represents the reducing sugars and is compared to a standard in a colorimeter.

When sucrose is to be determined 1 cc. of sugar solution is mixed with 2 cc. of saturated picric acid in a sugar tube and heated in boiling water 10 minutes to effect hydrolysis. One cubic centimeter of 20 per cent sodium carbonate is then added and the tube heated in boiling water for 20 to 30 minutes to develop the color. This color represents the total sugars. The difference between the two sets represents the sucrose present.

All color measurements were made in a Kober colorimeter.

The various items for which modifications are suggested are the color standard, the effect of picric acid on glucose, the dilution of the unknown color, clarification, the calculation of results, and the color factors for various sugars.

## THE COLOR STANDARDS

Several color standards have been suggested, but all of them have some disadvantageous feature. The picramate color does not match ferric acetate (8), potassium bichromate, or o-nitrophenolate (5). Dehn and Hartman (5) used the color produced by a standard glucose solution, while Lewis and Benedict (10) used pure picramic acid.

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<sup>1</sup> Received for publication Feb. 19, 1924. Published, with the approval of the Director, as Paper No. 428, Journal Series, Minnesota Agricultural Experiment Station.

<sup>2</sup> Thomas, W., and Dutcher, R. A. The application of the Benedict-Osterberg method to the quantitative estimation of carbohydrates in plant tissues. Paper presented at convention of Amer. Chem. Soc., Milwaukee, Wis., September, 1923. (Not published.)

<sup>3</sup> Willaman, J. J., Burr, G. O., and Davison, F. R. Cornstalk sirup investigations. In press.

<sup>4</sup> Reference is made by number (italic) to "Literature cited," pp. 487-488.



At first it was thought that a standard glucose solution in saturated picric acid might be a convenient source of color, since a standard tube could be prepared each time a series of analyses is run. It was found, however, that such a solution gradually increases in color-producing value, being about 115 per cent of its original value at the end of a year. The change is rather slow, however, and the solution may be used for about a week without any appreciable change being noticed. Solutions of pure picramic acid, or of sodium picramate, match the sugar colors exactly, but in the writers' experience they faded too rapidly to be satisfactory. It was thought that the change might be hastened to completion by treating with heat or with intense light or with both. But instead of fading, the color became more intense during the treatment, and then faded again, so this method proved useless. The usual method is to make a standard glucose solution each time a set of analyses is to be run. This is time-consuming but accurate, and is possibly the best procedure available at present, especially where analyses are not run daily.

Another procedure that was tried was to make the color in bulk from sugar, and then store in amber bottles. Without giving the data in detail, it is sufficient to say that the colors from both sucrose and glucose, made in quantities of 500 cc., and stored in both clear and in amber bottles, faded rapidly. After 30 days the fading was much slower, but it was still too marked even after 100 days to warrant using the material as a standard.

From the above experiences, the writers recommend as a standard an 0.08 per cent glucose solution in saturated picric acid, making a new solution each week. A reduction in sugar tubes is made with each set of analyses run, using 1 cc. of sugar, 1 cc. of water, 1 cc. of saturated picric acid, and 1 cc. of 20 per cent sodium carbonate solution. This is the equivalent of using 1 cc. of unknown sugar solution and 2 cc. of picric acid. This is the most convenient standard where daily analyses are run. Where the analyses are intermittent, it is simpler to make an 0.08 per cent glucose solution in water, and make reductions of it in the usual way.

### EFFECT OF PICRIC ACID ON GLUCOSE AND FRUCTOSE

It was mentioned above that glucose, on standing in picric acid at room temperature, gradually increases its chromogenic value. It was also stated that when sucrose is to be determined by the present method the solution of sugar is first heated for 10 minutes with the picric acid alone, for inversion, and then heated again with sodium carbonate, for reduction. (Rose (16) found 10 minutes was sufficient for inversion, and this has been corroborated by the writers.) It was noticed that sucrose always gave more than its theoretical color value. These facts suggested that either the heating in the presence of picric acid has some effect on the glucose and fructose, or the fructose constituent has a greater chromogenic value than the glucose. The latter alternative was proved not to be the case, as will be mentioned below.

It was then decided to investigate the heat effect. Okey (13) found that both glucose and fructose have enhanced color values when heated with picric acid, and she abandoned the attempt to use picric acid as a hydrolyst for inulin. A standard glucose solution was treated both ways: that is, the color developed both with and without the 10-minute heating with picric acid. These two treatments duplicate the procedure for total sugars and for reducing sugars, respectively. The results are shown in Table I. It is apparent that glucose gives a more intense color when subjected to a preliminary heating with picric acid. Heating for 10 minutes causes a maximum development of color. In further trials this color was found to be constant regardless of the ratio of reducing sugars to sucrose

present. Evidently the sucrose is inverted in a short time and the invert sugar produced is exposed to the picric acid for the greater part of the 10-minute period. Fructose was not tried alone, but sucrose gave quantitatively the same effect as glucose alone. Because of this, and because Okey got similar results, it was assumed that both glucose and fructose were affected in the same way by the picric acid. The ratio of the color values with and without the heating in presence of picric acid, or in other words the "total" to "reducing sugar" values, was determined, and found to be 1.045.

This difference in color development may be dealt with in two different ways in making analyses by this method: Either two standards may be used, one for "total" and one for "reducing" sugars, or one standard can be used for both and a factor applied. The latter method was decided upon and the factor 1.045 has been used throughout the present work.

TABLE I.—*Effect of heating on an 0.08 per cent glucose solution in picric acid before heating with sodium carbonate—comparison made with a standard 0.08 per cent glucose set at 30 mm.*

Time of heating, minutes.....	10	15	20	25	30
Colorimeter readings:					
Series I.....	28.7	-----	-----	-----	-----
Series II.....	28.6	-----	-----	-----	-----
Series III.....	28.8	28.8	28.4	27.9	28.6

### EFFECT OF DILUTING THE PICRAMATE COLOR

In analyzing an unknown material, a color too intense for reading in the colorimeter may be obtained when the solution is diluted in the conventional manner to 10 c.c. Therefore it was desired to know whether the colored solution could be diluted to other volumes without destroying the proportionality of intensity. Determinations were run on 0.64, 0.32, and 0.16 per cent glucose and the resulting colors made to 80, 40, and 20 cc. respectively. These were then compared with an 0.08 per cent glucose made to 10 cc. The results are shown in Table II. It is apparent that either the high ratio of sugar to picric acid, or the dilution itself, disturbs the proportionality rather seriously. Hence dilution cannot be used to remedy a color that is too strong for comparison, although this has often been recommended (2, 11, 12e). Dehn (4) lists this dilution effect among his fallacies of colorimetry.

### CLARIFICATION

The choice of a clarifying agent is of primary importance in any sugar method. In trying to choose the best one for the present method some surprising results were obtained, which tend to cast doubt on the necessity of clarification in the case of many materials.

Benedict (1) clarified blood from proteins with picric acid. Folin and Wu (6) used a tungstic acid preparation for clarification. Høst and Hatlehol (7) made a comparative study of blood sugar results and found the Folin-Wu method gave good results both on tungstate and picric-picrate filtrates; while the Benedict method gave high results with picric-picrate filtrates.

TABLE II.—*Effect of dilution on the color value of glucose solutions*

Glucose solution	Final volume	Colorimeter reading	Recovery
<i>Per cent</i>	<i>Centimeter</i>	<i>Millimeter</i>	<i>Per cent</i>
0.08	10.0	30.0	100
0.16	20.0	28.5	105
0.32	40.0	27.7	108
0.64	80.0	27.4	110

TABLE III.—*Comparison of clarification methods*

Material	Sucrose		Reducing sugar		Total sugar	
	Not clarified	Clarified	Not clarified	Clarified	Not clarified	Clarified
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
LEAD ACETATE						
Corn-stalk juice.....	5.50	5.53	3.15	3.28	8.65	8.81
Do.....	10.23	10.06	2.93	2.97	13.16	13.03
Do.....	5.14	5.11	2.96	3.04	8.10	8.14
Do.....	8.82	8.92	3.23	3.02	12.15	11.94
Do.....	6.62	6.70	2.93	2.97	9.55	9.67
Do.....	11.42	11.26	3.03	3.04	14.45	14.30
Apple juice.....			8.40	8.46		
Grape juice.....			17.80	17.66		
ALCOHOL						
Corn-stalk juice.....	5.43	5.42	3.36	3.37	8.79	8.76
Do.....	4.54	4.56	3.20	2.98	7.74	7.54
SODIUM TUNGSTATE						
Corn-stalk syrup.....			14.50	14.56		
Sorghum syrup.....			11.10	11.30		
Do.....			18.10	18.10		
Diastophore.....			14.50	14.40		
PICRIC ACID						
Sorghum syrup.....	37.26	39.33	34.92	32.04	72.18	71.37
Do.....	24.25	27.67	35.64	33.12	59.89	60.79

Table III shows the results obtained by various methods of clarification. When lead acetate was used deleading was accomplished with potassium oxalate.

Alcohol clarification of corn juice was obtained by heating 400 cc. of juice with 600 cc. of 95 per cent alcohol for 20 minutes in a water bath. The alcoholic filtrate was diluted 25 times with water and 1 cc. was used for a sample, without removing the alcohol.

Rumsey's directions (17) for clarification with sodium tungstate were followed, an acidity of approximately  $P_H$  2 being obtained with sulphuric acid. The filtrate was diluted 100 times and used for analysis without the removal of the excess tungstate. The "diastophore" is a malt extract preparation.

Picric acid clarification was obtained by saturating the sugar solution with crystals, allowing it to stand for a few minutes, filtering, and analyzing the filtrate immediately, so as to preclude the possibility of inversion of sucrose.

The results show in general that clarification is unnecessary with the picrate method with the materials so far analyzed. Undoubtedly the picric acid clarification could be effective only when proteins are present, and the latter are probably absent in the syrups analyzed. Therefore the above results do not necessarily preclude the desirability of using it in the case of other materials, as, for example, the vegetables analyzed by Myers and Croll (12).

Preparations which contain chlorophyll, and which have been preserved in alcohol, have to be clarified with lead after dilution, since the chlorophyll precipitates and must be removed. The writers suggest that, when a number of samples of a given material are to be analyzed with the present method, lead clarification be used on a few samples, and then, if no effect is noticed, that no clarification at all be used.

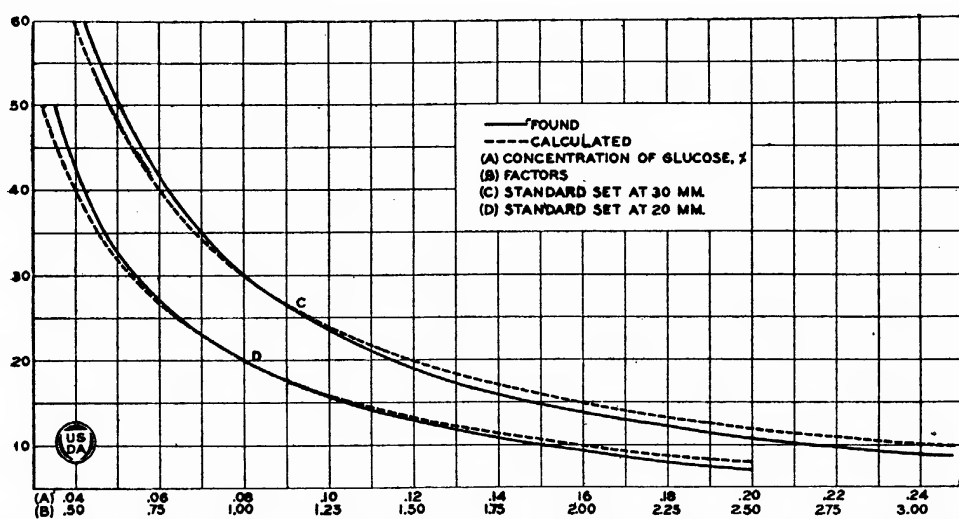


FIG. 1.—Color values of a series of glucose solutions from 0.02 per cent to 0.24 per cent, using the 0.08 per cent solution as a standard. From this graph the factors in Table IV were obtained

### CALCULATION OF RESULTS

It is generally assumed, in using a colorimeter, that the height of the column of liquid required to produce a given intensity is inversely proportional to the concentration of the colored substance, when there is not too great a difference in the intensity of the two solutions. Dehn (4) has pointed out the fallacy of this. For example, the writers found that, if two solutions of sugars are measured, one just double the strength of the other, and the weaker placed at 30 mm., the stronger reads 13.8 instead of 15. Conversely, if the stronger is set at 30, the weaker reads 64 mm. instead of 60 mm. The stronger is always too strong, and the weaker too weak. In practice, this discrepancy is met by the use of standards which have about the same intensities as the unknowns have. But this involves a series of standards. Kober (9) has a correction formula for nephelometry that applies in colorimetry also, but it was thought simpler to establish a table of corrections rather than to apply the formula to each calculation. The picramate color was prepared from glucose solutions varying in concentration from 0.02 to 0.24 per cent, the feasible range for the colorimeter used. The procedure for total sugars was used; that is, the glucose was heated for 10 minutes with the picric acid alone, and then 30 minutes with sodium carbonate. The 0.08 per

cent glucose solution was used as a standard and set at 30 mm. and at 20 mm. The readings were then plotted and curves drawn. From the curves a table was constructed showing the factor by which the percentage strength of the standard used is multiplied in order to obtain the concentration of the unknown solution at any reading on the colorimeter. The columns for reducing sugars were obtained by multiplying the "total" factors by 1.045. Figure 1 contains the curves from which the factors in Table IV were obtained. The dotted line in the chart represents the theoretical ratios of direct proportionality.

TABLE IV.—Factors for obtaining the "total" and "reducing" values of solutions for colorimeter readings from 7 to 60

Color- imeter read- ing	Total sugar		Reducing sugar		Color- imeter read- ing	Total sugar		Reducing sugar		Color- imeter read- ing	Total sugar		Reducing sugar	
	Standard set at—		Standard set at—			Standard set at—		Standard set at—			Standard set at—		Standard set at—	
	30	20	30	20		30	20	30	20		30	20	30	20
7.0	-----	2.49	-----	2.60	16.5	1.69	1.19	1.77	1.24	34.0	0.89	0.60	0.93	0.63
7.3	-----	2.42	-----	2.53	17.0	1.65	1.16	1.73	1.21	35.0	0.87	0.58	0.91	0.61
7.6	-----	2.35	-----	2.46	17.5	1.61	1.13	1.68	1.18	36.0	0.84	0.57	0.88	0.60
8.0	-----	2.28	-----	2.38	18.0	1.56	1.11	1.63	1.16	37.0	0.82	0.56	0.86	0.59
8.3	-----	2.21	-----	2.31	18.5	1.53	1.08	1.60	1.13	38.0	0.80	0.55	0.84	0.57
8.6	3.09	2.14	3.23	2.24	19.0	1.49	1.05	1.56	1.10	39.0	0.79	0.54	0.83	0.56
9.0	2.97	2.07	3.10	2.16	19.5	1.46	1.03	1.53	1.08	40.0	0.78	0.53	0.82	0.55
9.3	2.87	2.00	3.00	2.09	20.0	1.43	1.00	1.49	1.04	41.0	0.76	0.52	0.79	0.54
9.6	2.77	1.93	2.89	2.02	20.5	1.40	0.98	1.46	1.02	42.0	0.74	0.51	0.77	0.53
10.0	2.67	1.87	2.79	1.95	21.0	1.38	0.96	1.44	1.00	43.0	0.73	0.50	0.76	0.52
10.3	2.60	1.82	2.72	1.90	21.5	1.36	0.94	1.42	0.98	44.0	0.71	0.49	0.74	0.51
10.6	2.53	1.78	2.64	1.86	22.0	1.33	0.92	1.39	0.96	45.0	0.70	0.48	0.73	0.50
11.0	2.46	1.72	2.57	1.80	22.5	1.30	0.90	1.36	0.94	46.0	0.68	0.47	0.71	0.49
11.3	2.40	1.68	2.51	1.76	23.0	1.28	0.88	1.34	0.92	47.0	0.67	0.46	0.70	0.48
11.6	2.34	1.64	2.44	1.71	23.5	1.25	0.86	1.31	0.90	48.0	0.66	0.45	0.69	0.47
12.0	2.27	1.60	2.37	1.67	24.0	1.23	0.84	1.29	0.88	49.0	0.64	0.44	0.67	0.46
12.3	2.22	1.56	2.32	1.63	24.5	1.20	0.83	1.25	0.87	50.0	0.63	0.43	0.66	0.45
12.6	2.17	1.52	2.27	1.59	25.0	1.18	0.82	1.23	0.86	51.0	0.62	-----	0.65	-----
13.0	2.12	1.48	2.22	1.55	25.5	1.16	0.80	1.21	0.84	52.0	0.61	-----	0.64	-----
13.3	2.07	1.45	2.16	1.52	26.0	1.13	0.78	1.19	0.82	53.0	0.60	-----	0.63	-----
13.6	2.02	1.42	2.11	1.48	27.0	1.10	0.76	1.15	0.79	54.0	0.59	-----	0.62	-----
14.0	1.97	1.38	2.06	1.44	28.0	1.07	0.73	1.12	0.76	55.0	0.58	-----	0.61	-----
14.3	1.93	1.35	2.02	1.41	29.0	1.03	0.70	1.08	0.73	56.0	0.57	-----	0.60	-----
14.6	1.89	1.32	1.97	1.38	30.0	1.00	0.68	1.04	0.71	57.0	0.56	-----	0.59	-----
15.0	1.85	1.29	1.93	1.35	31.0	0.97	0.66	1.01	0.69	58.0	0.55	-----	0.57	-----
15.5	1.79	1.25	1.87	1.31	32.0	0.94	0.64	0.98	0.67	59.0	0.54	-----	0.56	-----
16.0	1.73	1.22	1.81	1.27	33.0	0.92	0.62	0.96	0.65	60.0	0.53	-----	0.55	-----

The necessity for a special set of factors to be used when the standard is set at 20 mm. was brought out when four color preparations were compared to an 0.08 per cent glucose standard set at 10, 20, 40, and 50 mm. The results are plotted in Figure 2. The dotted line represents the theoretical values which should be obtained if strict proportionality existed, calculated from the lowest reading of the standard in each set.

COLOR FACTORS FOR VARIOUS SUGARS

Since all reducing sugars give picramic acid when acting on picric acid, these various sugars can be determined by the present method, provided no interfering substances, either sugars or nonsugars, be present. It was therefore thought desirable to determine the chromogenic value of certain of the commoner sugars. Dehn and Hartman (5) give several of these, but not in the form of usable factors. The sugars used by the writers were of the highest purity, and met the specifications of Pfanstiehl and Black (14) for the rare sugars. The colors produced were compared to the color from a standard glucose solution. The procedure for

“reducing” sugars was used; the effect of heating these various sugars with picric acid was not determined. The values given in Table V are for anhydrous sugars, and are based on glucose as unity. They are the ratios of the color intensity of glucose compared to the intensities of equal weights of the other sugars.

TABLE V.—Color values of various sugars, compared with glucose as 1.000

Sugar	Color value	Conversion factor	Sugar	Color value	Conversion factor
Glucose.....	1.000	1.000	Rhamnose.....	1.298	0.774
Fructose.....	1.000	1.000	Lactose.....	0.750	1.333
Maltose.....	0.770	1.298	Arabinose.....	1.000	1.000
Mannose.....	1.000	1.000	Xylose.....	1.071	0.934
Galactose.....	0.883	1.132			

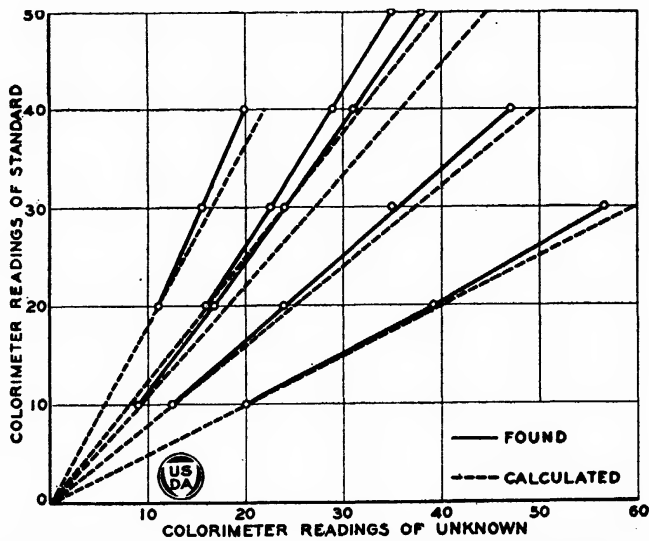


FIG. 2.—Curves showing the differences in color value obtained when the standard is set at 10, 20, 30, 40, and 50 mm.

In using this method on solutions of sugars other than those having a color value of 1.000, either a standard may be made from the pure sugar involved, or the ordinary glucose standard may be used; and, after calculating the sugar content in the usual manner according to the suggestions below, the number obtained is multiplied by the conversion factor given in the table for the particular sugar involved.

MINOR PRECAUTIONS

Rhode and Sweeney (15) and Benedict (1) have emphasized the importance of the purity of the picric acid used in this method, not only from the standpoint of its color-producing properties, but from that of its precipitating power towards proteins. The writers tried the various methods of purification of picric acid that have been suggested, and found practically no difference. Therefore simple recrystallization from hot water acidified with 15 cc. of concentrated hydrochloric acid per liter is recommended.

Benedict and Osterberg (2) pointed out the necessity of a proper proportion of picric acid to sugar. The writers found that varying the proportions does affect the intensity of color produced. They therefore emphasize the necessity of using

exactly 1 cc. of unknown sugar solution, 2 cc. of saturated picric acid, and 1 cc. of 20 per cent sodium carbonate.

During the 30 minutes of heating in a water bath, evaporation of water from the sugar tubes may be prevented (1) by loosely inserting corks covered with tinfoil in the tops of the tubes; and (2) by keeping the level of water in the bath approximately at the same level as the solutions in the sugar tubes. A wire rack fitting into a basin is a very convenient holder for the tubes.

Thus it will be seen that the conditions involved in determining sugar by this method are rather empirical. The writers believe that extreme empiricism is the only feasible basis for any colorimetric method. A given procedure and set of conditions must be decided upon, and then rigidly adhered to, whether they give maximum color intensities or not. The unknown color will be exactly comparable to the known, since they were both produced under exactly the same conditions. From this standpoint the most significant points in the present method are the effect of heat and picric acid on glucose, the use of the table of factors, and the use of exact amounts of reagents.

## PROPOSED METHOD IN DETAIL

### SOLUTIONS

1. *Saturated picric acid* that has been recrystallized from hot dilute hydrochloric acid.
2. Twenty per cent *sodium carbonate* (anhydrous).
3. An 0.08 per cent *standard glucose solution*, either in water or in saturated picric acid, as discussed above. An 0.076 per cent sucrose solution is equally suitable, and has the same color value.

### PREPARATION OF MATERIAL

If it is known that lead clarification has no effect on the results with the sugar solution in question, no clarification is needed. Otherwise the usual lead acetate clarification should be performed, followed by deleading with disodium phosphate. The solution is then diluted until both the sucrose and the reducing sugars are between 0.02 and 0.24 per cent.

### REDUCTION

1. *Reducing sugars*.—One cubic centimeter of the diluted sugar solution is pipetted into a sugar test tube, followed by 2 cc. of picric acid and 1 cc. of sodium carbonate. The solutions are thoroughly mixed and the tube lightly stoppered with a cork covered with tinfoil. The tubes are then immersed in boiling water for 20 to 30 minutes. They are then cooled and the contents diluted to the 10 cc. mark and shaken.

2. *Total sugars*.—One cubic centimeter of the diluted sugar solution and 2 cc. of picric acid are pipetted into a sugar test tube and immersed in boiling water for 10 minutes. Then 1 cc. of sodium carbonate solution is added, the mixture shaken, and the heating continued for 20 to 30 minutes. Cooling, mixing, and diluting are then conducted as stated above.

3. *Standard color*.—One cubic centimeter of the standard glucose or sucrose solution is treated the same as for *total sugars*.

## COLOR MEASUREMENT

The zero point of the colorimeter is adjusted, using the standard color solution on both sides. The *reducing* and *total* colors are measured against the standard set at 30 mm. or at 20 mm.

## CALCULATION

$$R = f_r \times \text{Std.}$$

$$T = f_t \times \text{Std.}$$

$$S = 0.95 (T - R).$$

$$T' = R + S$$

Where

$R$  = per cent of reducing sugars in the solutions analyzed.

$S$  = per cent of sucrose in the solution analyzed.

$T$  = per cent of total sugars (apparent) in the solution analyzed.

$T'$  = per cent of total sugars (true) in the solution analyzed.

$f_r$  = the factor obtained from Table IV, corresponding to the colorimeter reading for the *reducing* sugars, and to the position of the standard (30 or 20 mm.) for reducing sugars.

$f_t$  = the factor obtained from Table IV, corresponding to the colorimeter reading for the *total* sugar and to the position of the standard (30 or 20 mm.) for the *total* sugar.

Std. = value of the standard in terms of per cent of glucose.

## SUMMARY

1. A search for a reasonably permanent color standard for use with the picric acid method for sugars has been unsuccessful. The best standard is either an 0.08 per cent glucose solution, or an 0.076 per cent sucrose solution, in saturated picric acid, which will keep for about a week; or these same solutions in water, in which case they must be used at once.

2. Heating glucose in a solution of picric acid previous to reduction in a sodium carbonate solution gives a greater color value than without the treatment.

3. When the picramate color is diluted, the intensity of the color is not proportional to the dilution. Therefore the color is always diluted to exactly 10 cc.

4. Clarification of sugar solutions has proved to be unnecessary in a number of cases when the picramate method was used.

5. The intensity of the picramate color is not proportional to the amount of sugar present. Therefore a table has been compiled which gives factors to be used throughout the range of colorimeter readings.

6. The color values for 8 reducing sugars, in comparison with glucose, have been determined.

7. The necessity for strict empiricism in this method is emphasized, and the method in detail is given, involving the modifications presented in this paper.

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# EFFECT OF MOISTURE ON A SEED-BORNE BEAN DISEASE<sup>1</sup>

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## INTRODUCTION

In a preliminary paper (9)<sup>2</sup> attention was called to a wilt disease of navy beans, *Phaseolus vulgaris* L., noted at Redfield, S. Dak., which seemed to arise from the application of legume bacteria culture to the seed prior to planting or to be favored by such application. Experimental work at Redfield in 1922 indicated that inoculation applied in the liquid form, or water applied in a similar manner to the progeny of the seed on whose plants the original observations were made, stimulated the disease to such an extent that it was possible to superficially differentiate the rows so treated from those untreated. It was concluded, therefore, that moisture was largely responsible for these differences: The initial stimulation afforded by this means to the disease organisms in and on the seed being sufficient to cause an appreciable effect which was in evidence practically the whole time during the vegetative period and was reflected in the harvest of beans.

Moisture is artificially applied to many legume seed by inoculation with suspensions of nitrogen-fixing legume bacteria in water or nutrient solutions. Inoculation is not ordinarily a soaking process, although soaking has been recommended for certain legume seed; it is merely a case of light moistening generally followed by drying in air or immediate sowing. When carried out in the regular manner it will be very rare that stimulation of destructive bacteria or disease organisms will be perceptible. In the great majority of cases treatment with inoculation has been so decidedly beneficial that when a case to the contrary arises it is advisable to trace it to its cause.

## REVIEW OF LITERATURE

### FUNDAMENTAL EFFECTS OF SEED SOAKING

The effect of moisture on seed has been studied by many investigators and the literature concerning most of the studies is covered by Kidd and West. (8) The outstanding earlier work has been summed up by Wollny. (14) His general conclusion is that the harm occasioned by soaking in water was due to the loss of necessary soluble nutrients from the seed by osmotic transfer. This condition, it was found, is encouraged especially by excess water and is influenced by the species of seed, duration of the immersion, and the temperature of the water. Hiltner (7) found that long continued soaking in excess water reduced the disease-resisting power of the seed, encouraged the activities of destructive bacteria, but that moisture applied in such a manner as to simulate natural conditions had a beneficial effect. Kidd and West (8) using bean seed presumably free from disease which had been soaked for six hours in an excess of water at temperatures of from 5 to

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<sup>1</sup> Received for publication Feb. 27, 1924.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 496-497.

20° C., inclusive, found that a germination test indicated no injury, but in the subsequent growth it became evident that the plants from the soaked seed were less in size and weight than an equal number of plants from untreated seed.

Braun (1) by soaking wheat seed for 10 minutes in water, then draining and covering them for six hours, found that the efficiency of the disinfectant used was increased; due to the stimulation of the dormant bacteria on the seed into a growing condition, in which state they are more easily destroyed and that the harmful effects of long continued soaking were thus largely obviated. In his general work on the internally borne seed disease Chen (2) used this same method to free the seed coats from live organisms by disinfection.

#### DISTINCTION BETWEEN BLIGHT AND WILT

The disease to which this paper makes especial reference is produced by *Bacterium flaccumfaciens* Hedges and has been called bean wilt. Unfortunately the earlier literature makes no distinction between the bean disease caused by this organism which was only recently isolated and named, and the bean blight by *Bact. phaseoli* Erw. Sm. The statements in this brief review of the literature will apply to either or both of the diseases and the term blight will be applied to the disease not specifically ascribed to *Bact. flaccumfaciens*. As the two diseases mentioned are very closely allied and likely to be confused, it might be well to call attention to the differences between them as recently brought out by Florence Hedges (5, 6):

1. The wilt organism apparently does not produce stomatal infection as does the blight organism and is not accompanied by conspicuous yellowing of the leaves.

2. Wilt organisms resist desiccation for a much longer period (even 5 years) than that previously reported for *Bact. phaseoli*.

3. In pod infections the wilt follows the sutures whereas *Bact. phaseoli* produces spots.

#### FACTORS AFFECTING THE BEAN BLIGHT

With the idea of disinfection Edgerton and Moreland (3) treated bean seed infected with the blight organism by soaking for eight minutes in water at 50° C. They found that this treatment not only did not affect the blight organisms seriously but that the plants from the seed thus treated were more affected by the disease than the plants from the untreated seed. This anomalous condition they explained was probably due to the reduction of the vitality of the seed by the treatment, this contributing to the susceptibility of the plants to the disease. It is quite possible too that the stimulation of the seed-borne organisms occasioned by the water was an important factor. If so small an amount as is added by the process of inoculation can produce noticeable results, the moisture which naturally comes in contact with the seed must have a bearing on the development of the disease. Sackett (12) is of the opinion that warm wet weather favors the bean blight and Muncie (10) making observations in Michigan found that the disease is more widespread under wet and muggy conditions. Rapp (11) observed that cool wet weather does not favor the development of the disease but found that it continues to spread following warm wet weather. He considers rain and dew important factors in the transmission of the disease. Halsted (4) correlated an increase in bean blight to irrigation, and Sackett (13) thinks this is one of the factors for disseminating the disease.

#### THE RESULTS OF 1922

Since the harvest of seed from the 1922 experiments has not been reported hitherto, it seems advisable to give the results here (Table I) as a corroboration

of the evidence concerning the presence of wilt symptoms among the bean plants previously reported (9). From a practical standpoint, the amount of seed produced constitutes a better index of the effect than observations on the numbers of diseased plants.

The effect of the disease and moisture additions on the Redfield beans, it is revealed in the figures, is reflected also in the bean yields and the differences follow the same general trend that was indicated by observations on the growing beans, that is, the plants from seed treated with moisture gave a higher percentage of disease and a lower yield of seed than the plants from seed not treated with moisture.

On the other hand the clean seed shows the same correlation only in a minor degree. The plants from the selected seed acted more as a unit, all ripening their seed at approximately the same time whereas the plants from the Redfield seed ripened unevenly; at the time of harvest green beans and flowers were noticeable to the approximate extent of 10 per cent in all rows except one of those planted to untreated seed, which for some unknown reason ripened uniformly. There was evidence of the wilt among these immature beans.

Considering the average yield of 671 gm. as 100 per cent, the average reduction in crop, apparently due to the addition of moisture to the seed before planting, is 30.3 per cent, ranging from 51.3 per cent for the water treatment to 6.9 per cent reduction from treatment with bean culture No. 162.

TABLE I.—*Navy bean yields from plats at Redfield, S. Dak., in 1922*

Treatment <sup>a</sup>	Redfield seed <sup>b</sup>	Clean seed <sup>c</sup>
	Grams	Grams
None.....	710	809
Water.....	237	710
Ashby broth.....	525	802
Soil broth.....	511	767
Inoculated soil.....	632	824
Commercial culture.....	405	752
407 Wisconsin culture.....	469	724
342 United States Department of Agriculture culture.....	412	824
162 United States Department of Agriculture culture.....	625	767

<sup>a</sup> With the exception of the inoculated soil, all treatments were applied in the liquid form at the rate of approximately 10 cc. per 500 gm. of beans.

<sup>b</sup> Harvested Aug. 24, 1922.

<sup>c</sup> Harvested Aug. 19, 1922. Each figure is the average of two plats.

## EXPERIMENTAL WORK IN 1923

### PREPARATION AND PLANTING

The experience of 1922 led to the planning of more extensive work for 1923 under dry land, humid, and irrigated soil conditions. It was hoped to determine the conditions favorable for the development of disease in connection with inoculation with legume bacteria, the extent of these conditions, and additional proof of the efficacy of the measures for control.

The seed employed in the major portion of the experiments were progeny of the Redfield seed used in the former experiment. At Mandan, N. Dak., and Rosslyn, Va., a badly diseased mixture of commercial bean seed was used. Seed of Robust bean, cowpeas, and tepary beans were also planted with the idea of establishing the presence of disease amongst them and the stimulation of such disease if present by the addition of moisture. At each place the beans were planted in equal length rows, but owing to variations in the sizes of plots at the different places, the rows of the different plots are not comparable. As far as possible, rows were made equivalent to one four-hundredth of an acre. Each of the treatments given below was duplicated at each place.

- No. Treatment
- 1-----Liquid pure culture applied as usual.<sup>3</sup>
- 2-----No treatment.
- 3-----Liquid pure culture applied in excess and allowed to stand one hour before spreading out to dry.
- 4-----Dry soil applied by the glue method.<sup>4</sup>
- 5-----Dry soil applied by the glue method dried 10 days previous to planting.
- 6-----Liquid pure culture applied as usual, dried 10 days before planting.
- 7-----Dry soil applied by sowing in rows with seed.
- 8-----Liquid pure culture applied as usual dried 24 hours before planting. \*
- 9-----No treatment.
- 10-----Liquid pure culture applied as usual.

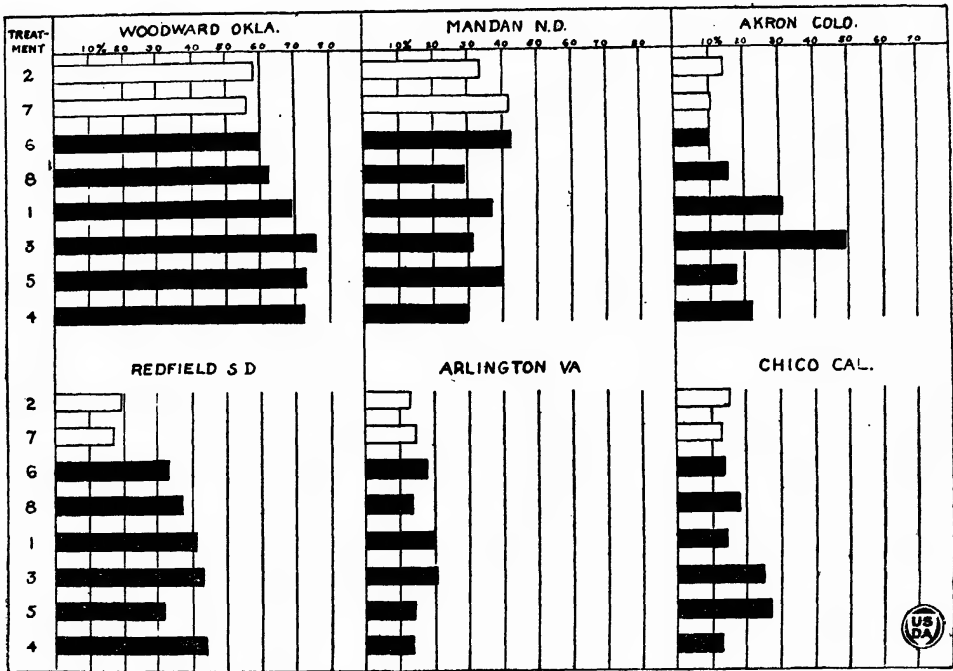


FIG. 1.—Chart showing percentage of observed wilt disease symptoms among navy beans under the different treatments. Entirely black bars represent the moisture treated rows

The same plan of experiment was conducted at the Great Plains stations of the Office of Dry Land Agriculture at Mandan, N. Dak.; Akron, Colo.; Woodward, Okla.; and at the station of the Office of Forage Crop Investigations, Redfield, S. Dak. It was conducted under irrigated soil conditions at the farm of the Office of Foreign Seed and Plant Introduction at Chico, Calif., and under humid conditions at the Arlington Experiment Farm of the United States Department of Agriculture at Rosslyn, Va., and on private ground at Leeland, Montgomery County, Md. Conditions at these places are indicated in Table II.

Liquid pure culture was applied at the approximate rate of 10 cc. per 500 gm. of bean seed, mixed with the seed by hand and at once spread out to dry. The excess application was made similarly with double the quantity of liquid, with the exception that the seed were covered with paper one hour before being spread out to dry.

<sup>4</sup> A 5 per cent glue solution is prepared by adding furniture glue to boiling water and cooling. The seed are moistened with this mixture and dry infected soil sifted over them to absorb the moisture and adhere to the seed coats.

## PRESENCE OF WILT SYMPTOMS

Observations were made on the experiments by counting all of the plants in a row and also the plants which gave evidence of wilt disease symptoms or were dead either on account of drought, injury, or other disease.

The effect of drought on the mortality among the beans at Woodward, Okla., is shown in Figure 1. If we consider this a constant factor the results may still have a meaning as regards the effect of the combination of moisture and disease.

TABLE II.—*Soil types, initial moisture and dates of planting beans at the various places of experiment*

Place of experiment	Type of soil	Initial moisture <sup>a</sup>	Date of planting	Date of observation
		<i>Per cent</i>		
Arlington, Va.....	Clay loam.....	11	June 15, 1923	Sept. 10, 1923
Akron, Colo.....	Light sandy loam.....	10.7	May 18, 1923	Aug. 4, 1923
Chico, Calif.....	Sandy loam.....	7.9	May 3, 1923	Aug. 10, 1923
Leeland, Md.....	Chester loam.....	6.4	June 26, 1923	
Mandan, N. Dak.....	Black sandy loam.....	2.8	May 23, 1923	Aug. 21, 1923
Redfield, S. Dak.....	Black clay loam.....	7.9	May 26, 1923	Aug. 24, 1923
Woodward, Okla.....	Canadian sandy loam.....	4.9	May 12, 1923	Aug. 1, 1923

<sup>a</sup> Determinations made by Franklin W. Marsh of the Office of Soil Bacteriology, United States Department of Agriculture

At Redfield and Woodward, there is a consistent increase in the amount of disease corresponding to increasing applications of moisture. Excluding the data from Mandan there is a general indication of the same result at all other places but it is less striking than at Woodward and Redfield. Nodules were present on roots at all places.

Among the other legumes used only the Robust beans showed occasional symptoms of the wilt.

## SEED HARVEST

The seed were harvested at all places except Woodward and Akron. They were examined for the presence of diseased and abnormal seed. The figures in Table III give in detail the yields from the various places together with the results of the pickage.

The average of the effect of the moisture treatments are compared with the control and dry treatments in Figure 2. These are shown together. It is significant that in each case the crops from the seed planted dry exceed those of the moisture treatments.

## DISCUSSION

## EFFECT OF RAINFALL AND TEMPERATURE

To determine whether a correlation existed between the extent of the disease and the rainfall a study has been made of the records from Redfield and other places at which the experiments were made. (fig. 3).

To give an idea of the condition of the soil at Redfield as regards moisture, rainfall prior to planting has been considered. The period covered following the planting is the approximate time of vegetative growth for navy beans. If we consider the first three years only, it will be noticed that in 1920, when the damage was the greatest, the rainfall was also excessive, but if the record of 1923, also a rainy year, is included this deduction is vitiated by the fact that the damage in this year can not be considered excessive.

A review of the rainfall data in 1923 at the various places where the experiments were made does not indicate great differences in total or periodical rainfall between the dry-land and humid sections. At Chico, Calif., the rainfall was slight, but it was supplemented by irrigation at six different times during the season. There seems to be no correlation between the moisture in the soil at planting time and the subsequent amount of disease.

If the temperatures of 1920 and 1923 at Redfield are compared (fig. 4) it is found that when the plantings were made, there was a difference of 8°, 1923 having the higher temperature. Throughout the growing period there was a rather regular alternation of high and low temperatures during both years. The rise and fall periods were approximately 10 days in length and were quite noticeable for the first 50 days. The mean daily temperature of 1920 for the period is 67 $\frac{3}{4}$ ° F., whereas that for 1923 is 70 $\frac{3}{4}$ ° F. Taking temperature, moisture, and the observations of previous investigations into consideration, it was reasonable to expect that the damage among the beans would be greater in 1923.

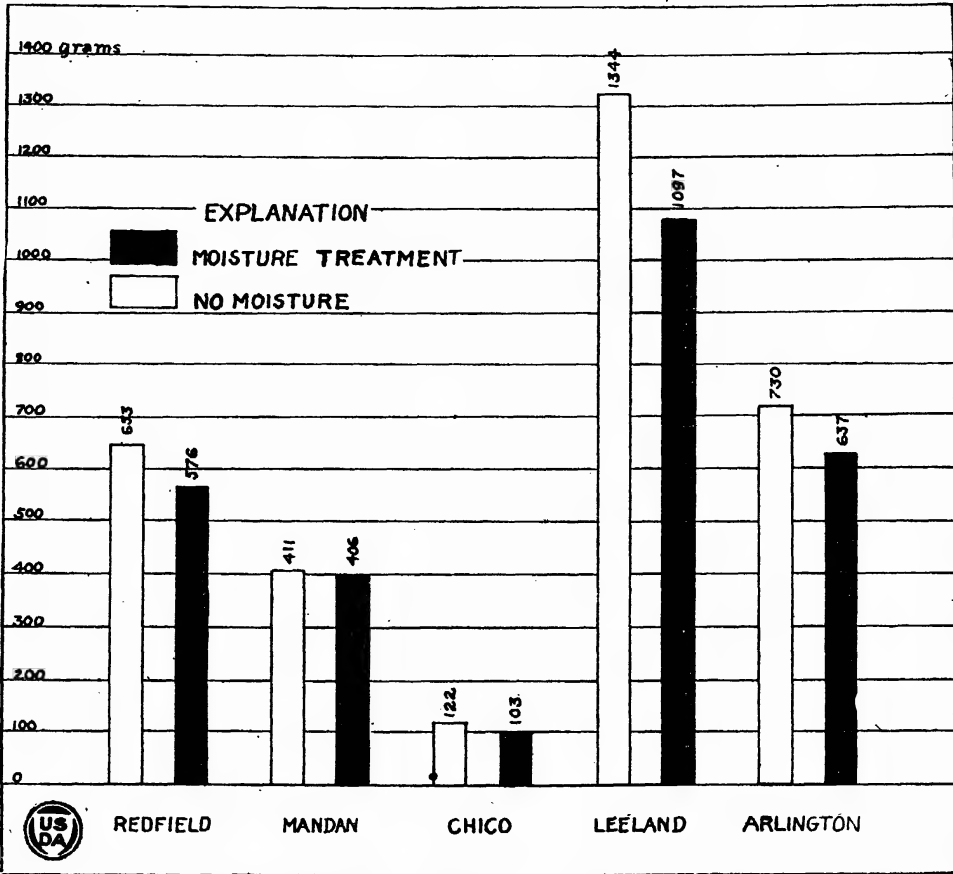


FIG. 2.—Chart showing graphically the average differences in yield in grams between the moisture and no moisture treatment

Such, however, was not the case. It is probable, therefore, that moisture added to the seed before planting is a more potent factor in the stimulation of the bean disease than the natural conditions which obtain during the life of the plant.

TABLE III.—Seed yields, in grams, from 1923 bean plots

Treatment	Redfield, S. Dak.			Mandan, N. Dak.			Chico, Calif.			Leeland, Md.			Arlington, Va.		
	Total yield	Clean seed	Per cent diseased	Total yield	Clean seed	Per cent diseased	Total yield	Clean seed	Per cent diseased	Total yield	Clean seed	Per cent diseased	Total yield	Clean seed	Per cent diseased
None..	622	543	12.7	440	391	11.2	137	128	6.6	1,362	1,073	21.2	704	534	24.2
1.....	512	422	17.6	462	422	8.7	81	76	6.2	982	775	21.1	641	550	14.2
3.....	481	402	16.4	445	405	9.0	111	109	1.8	1,098	852	22.4	563	453	19.6
6.....	729	604	17.1	277	244	11.9	120	106	11.7	1,113	906	18.6	761	601	21.0
8.....	637	480	24.7	372	338	9.2	71	64	9.9	1,217	1,023	16.0	720	559	22.4
7.....	684	604	11.7	382	352	7.9	107	98	8.4	1,326	1,091	17.8	757	580	23.4
4.....	436	384	12.0	511	461	9.8	106	100	5.7	1,050	880	16.2	558	428	23.3
5.....	662	529	20.1	370	322	13.0	130	125	3.9	1,124	907	19.3	580	479	17.4

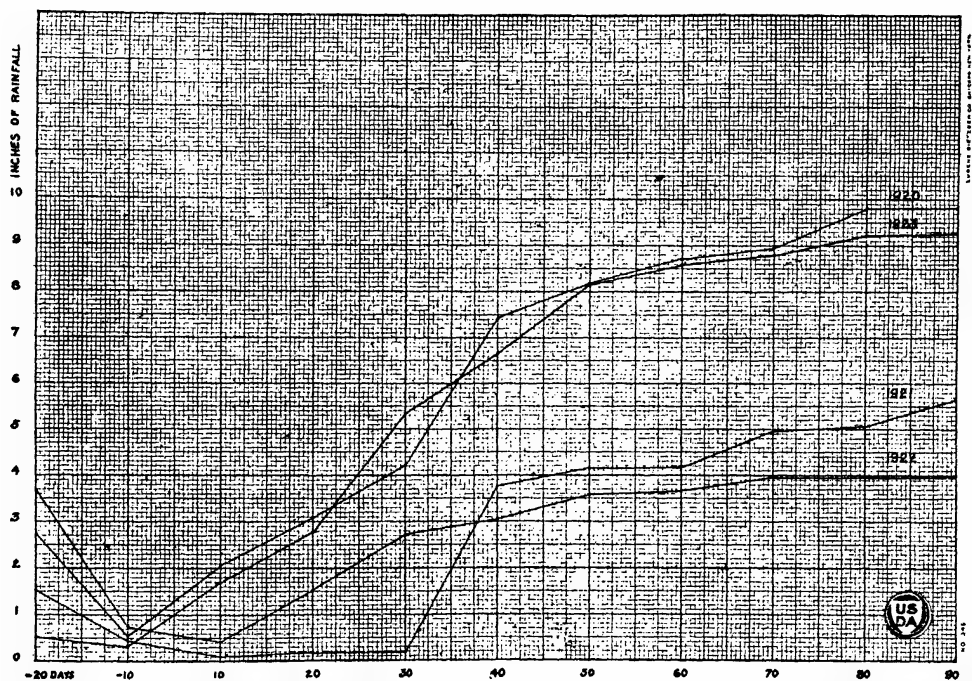


FIG. 3.—Graph showing accumulative rainfall data from 20 days prior to planting to 90 days after planting for four years at Redfield, S. Dak.

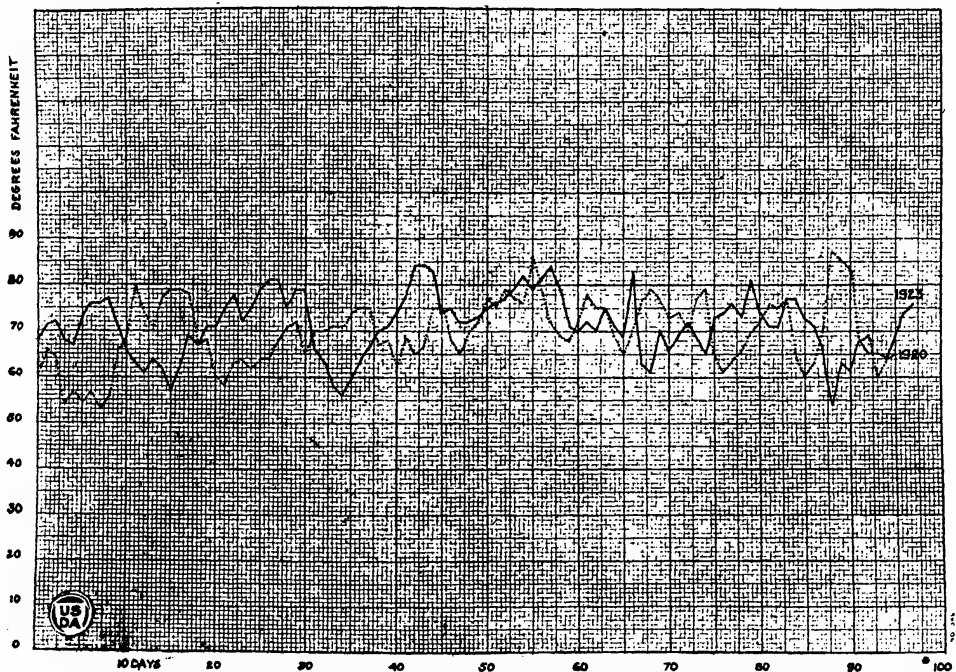


FIG. 4.—Graph showing mean daily temperatures from the time of planting at Redfield, S. Dak., for the years 1920 and 1923



## ECONOMIC ASPECT

Observations made on legume inoculation experiments in different parts of the United States over a period of 20 years have shown that only in extremely rare cases has the effect of legume bacteria cultures been apparently detrimental to the crop. The intent of this paper is not to emphasize an imminent danger but to record the possibilities which may under certain circumstances be expected to arise.

Recalling that the estimated loss from wilt at Redfield in 1920 was 90 per cent; in 1921, 25 per cent; in 1922, 30 per cent; in 1923, 12 per cent; and of the crop that the average loss from all places where the bean experiments were harvested in 1923 was approximately 11 per cent, that the Plant Disease Survey Bulletin of the United States Department of Agriculture gives for the loss to the total crop from blight for three years starting with 1920, as 1.5 per cent, 9.8 per cent, and 4.9 per cent, respectively, and that Muncie estimates that 30 per cent of the bean pickage is infected with the blight, the possibilities for damage become apparent. It seems reasonable to assume that the same general effect that has been produced by bean wilt in the work under discussion may also occur with other seed borne diseases.

The data obtained from the clean seed in 1922 and from clean Robust beans in 1923 indicates rather clearly the value of such seed for planting and the correctness of the common recommendations for its use.

## SUMMARY

A general tendency toward reduced crops of navy beans, due to applications of moisture to seed presumably harboring *Bact. flaccumfaciens*, prior to planting, is indicated by the harvests from the various places of experiment.

Dry soil as inoculating material will obviate the necessity for moistening the seed for the purpose of introducing *Bacillus radicola* and clean seed will tend to cut down losses even when moisture is applied.

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# JOURNAL OF AGRICULTURAL RESEARCH

## CONTENTS

	Page
Factors Influencing the Binding Power of Soil Colloids - - - - -	499
HOWARD E. MIDDLETON	
The Genetic Relation between <i>Triticum dicoccum dicoccoides</i> and a Similar Morphological Type Produced Synthetically - - - - -	515
H. H. LOVE and W. T. CRAIG	
Bud Selection as Related to Quality of Crop in the Washington Navel Orange - - - - -	521
A. D. SHAMEL, C. S. POMEROY, and R. E. CARYL	
The Determination of Nitrate and Ammonia in Nitrogenous Materials - - - - -	527
O. M. SHEDD	
<i>Puccinia graminis poae</i> Erikss. and Henn. in the United States - - - - -	541
E. C. STAKMAN and M. N. LEVINE	
Relation of Soil Temperature and Soil Moisture to Infection by <i>Plasmodiophora brassicae</i> - - - - -	549
JOHN MONTEITH, Jr.	
Length of Cotton Fiber from Bolls at Different Heights on the Plant - - - - -	563
THOMAS H. KEARNEY and GEORGE J. HARRISON	
"Hairy Neck" Wheat Segregates from Wheat-Rye Hybrids - - - - -	567
CLYDE E. LEIGHTY and J. W. TAYLOR	
<i>Ascaris</i> Sensitization - - - - -	577
B. H. RANSOM, W. T. HARRISON, and J. F. COUCH	
<i>Physalospora malorum</i> on Currant - - - - -	583
NEIL E. STEVENS	
<i>Botryosphaeria</i> and <i>Physalospora</i> on Currant and Apple - - - - -	589
C. L. SHEAR, NEIL E. STEVENS, and MARGUERITE S. WILCOX	
Isolation of an Inhibitory Substance from Plants - - - - -	599
W. L. MALLMANN and CARL HEMSTREET	
Two Hitherto Unreported Diseases of Stone Fruits - - - - -	603
C. C. LINDEGREN and DEAN H. ROSE	

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## FACTORS INFLUENCING THE BINDING POWER OF SOIL COLLOIDS<sup>1</sup>

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### INTRODUCTION

In a previous publication by Moore et al. (7),<sup>2</sup> of this Bureau, the point was brought out that the colloidal material is evidently the principal binding material of the soil. Further study has not only confirmed this point but, as shown by Anderson et al. (1), approximately 95 per cent of the total absorption of the soil is due to the soil colloids. The influence of the soil colloids on the physical and chemical properties of soil is no doubt coming to be one of the most important studies in soil science. The binding power of the soil colloids is of particular importance as regards tillage operations, foundations, and the construction of dams and roads. With these points in mind this investigation was undertaken in order to determine if possible the factors influencing the binding power of the soil colloids and the relation of the compressive strength of the soil to the amount of colloidal material present in the soil.

### PREVIOUS WORK

Various methods for the determination of the binding power of soils have been described. The methods of Atterberg (3) and Puchner (8) are probably the more important. These methods are based primarily on the breaking strength of briquettes. Marquis (6) describes various methods for determining the binding power of soils, particularly those of Atterberg and Puchner. He found very wide discrepancies in the results by each of these methods. More recently Arnd (2) has described a method by which he determines the binding power by friction, rotating a cylinder of soil against a stationary prism of soil under a definite pressure. None of these methods, however, takes into account the colloidal content of the soil.

Jackson (5) has described a method for determining the binding power of rock powder, which was modified somewhat and used in this investigation.

### METHOD OF INVESTIGATION AND APPARATUS

For the purposes of this investigation the material<sup>3</sup> under consideration was made up into briquettes 25 mm. in diameter and 25 mm. high under a pressure of 2,000 pounds per square inch, with a certain predetermined amount of water, carefully dried, heated 18 hours at 110° C., cooled in a desiccator and subjected to the compression test in an Olsen universal testing machine.<sup>4</sup> The load

<sup>1</sup> Received for publication March 24, 1924.

<sup>2</sup> Reference is made by number (*italic*) to "Literature cited," p. 513.

<sup>3</sup> "Material" as used in this paper refers to soil or to mixtures of soil colloids and sand.

<sup>4</sup> The use of this machine was granted for this purpose by the Division of Tests, Bureau of Public Roads, U. S. Dept. of Agriculture.

indicated by the machine at the point of failure of the briquette was taken as its breaking strength. The average of three or four such determinations was taken in each instance. The dry weights of the briquettes varied considerably with different materials; so for comparative purposes, the average load was divided by the weight of the briquette, giving the load per gram of material. By dividing the load per gram of material by the amount of colloid in one gram of the material, the load per gram of colloid was determined.

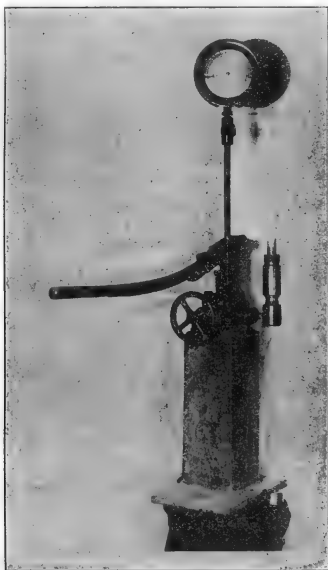


FIG. 1.—Hydraulic pump used in molding briquettes

For the purpose of forming the briquettes a hydraulic pump (fig. 1) was used. A brass cylinder (fig. 2) was drilled with a  $31/32$ -inch drill, bored to  $63/64$  inch and lapped out with a lead lap. This made the diameter of the opening 25 mm. A steel piston was turned out so that it slipped easily into this opening and was made water tight at its lower end with a leather washer. A small auxiliary cap was made to fit the opening at the top of the cylinder and a larger cap made to screw on over this. The purpose of the small cap was to protect the surface of the briquette when the outer cap was removed. The cylinder was attached to the pump, as shown in figure 1.

In making a briquette the piston was pushed to the bottom of the cylinder with a wooden stick, the material was introduced into the cylinder, and the caps put in place. The valve on the pump was closed and the pressure gradually raised to 2,000 pounds, as indicated by the gauge. After the pressure had been applied a sufficient length of time, it was released by opening the valve and the caps were removed. Then the valve was closed and the briquette pushed out of the cylinder (as shown in fig. 2) by operating the hydraulic pump, and removed.

All briquettes which were used for compression tests were made 25 mm. high. In order to determine the amount of material necessary to make a briquette 25 mm. high, a preliminary briquette containing 20 gm. of the material was made up. The height of this briquette was measured with a micrometer and the amount of material necessary to make a briquette 25 mm. high calculated by simple proportion. This preliminary briquette was then used to determine the amount of moisture in the material at the time the briquettes were made.

### GENERAL CONSIDERATIONS

At the beginning of this work, it was found very difficult to get determinations of the breaking strength of any one given material which could be duplicated at two different times. The briquettes in a given series made up at one time would give very different results from the same series made up at another time. In tracing out the causes of the discrepancies there were found to be four main factors governing the compressive strength of the briquettes, as follows:

(a) The amount of moisture present in the material when the briquettes were made.

(b) The treatment of the material before the briquettes were made.

(c) The pressure used in making up the briquettes.

(d) The method of drying the briquettes.

When these factors were taken into consideration the results could be duplicated within 5 per cent of the average load.

### MOISTURE CONTENT

Dry briquettes of a given material varied in breaking strength depending on the moisture content of the material. By varying the moisture content and keeping the dry weight of the material constant a point was found where the briquette reached a minimum size. This indicated that the density of the material was greatest at this point. For the purpose of this investigation, the moisture content at this point was called the critical moisture content. Since the diameters of the briquettes were held constant by the size of the opening in the cylinder, the differences in the size of the briquettes were indicated by their heights.

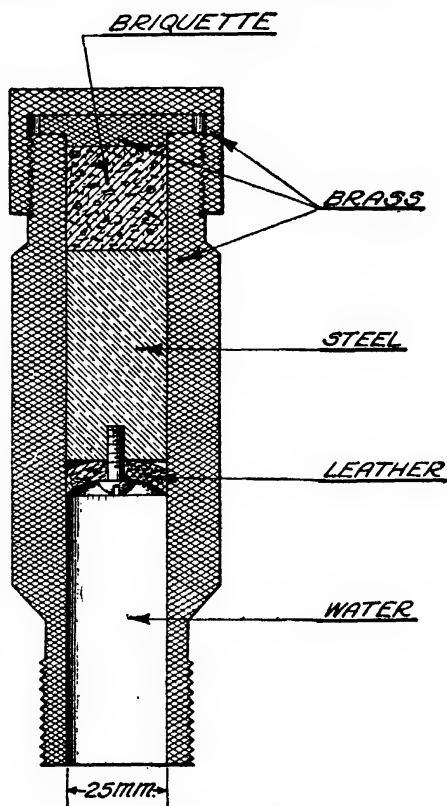


FIG. 2.—Cylinder for molding briquettes



TABLE I.—*Effect of the amount of moisture on the height of the briquette*

Soil	Weight of wet briquette	Weight of dry briquette	Per cent moisture	Height of briquette	Height of briquette per gram of dry soil
	<i>Grams</i>	<i>Grams</i>		<i>Milli-meters</i>	<i>Milli-meters</i>
Norfolk fine sandy loam soil.....	20.95	20.00	4.8	23.27	1.163
Do.....	21.48	19.99	7.5	22.75	1.138
Do.....	21.99	20.02	9.8	22.47	1.122
Do.....	22.47	20.03	12.2	22.50	1.123
Do.....	22.75	20.11	13.1	22.85	1.136
Marshall silt loam soil.....	20.97	19.90	5.4	26.60	1.337
Do.....	20.96	19.45	7.8	25.03	1.287
Do.....	21.96	19.93	10.2	24.74	1.242
Do.....	22.37	19.82	12.9	23.96	1.210
Do.....	22.95	19.99	14.8	23.75	1.189
Do.....	23.45	19.92	17.7	24.15	1.212
Do.....	23.94	19.90	20.3	25.27	1.270
Sharkey clay soil.....	21.78	20.00	8.9	26.63	1.332
Do.....	22.53	19.93	13.0	24.71	1.240
Do.....	23.12	19.95	15.9	23.95	1.200
Do.....	23.57	19.95	18.1	23.88	1.196
Do.....	23.78	19.92	19.4	24.17	1.213
Do.....	24.34	19.94	22.1	25.17	1.262

Table I shows how the height of the briquette varies with the amount of moisture for three different soils. In order to determine the critical moisture content the height per gram of dry soil is plotted against the per cent moisture as in figure 3 and the value at the lowest point of the curve indicates the critical moisture content.

TABLE II.—*Effect of the amount of moisture on the breaking strength of the briquette*

Soil	Moisture	Weight of briquette	Average load per briquette	Load per gram of soil
	<i>Per cent</i>	<i>Grams</i>	<i>Kilograms</i>	<i>Kilograms</i>
Norfolk fine sandy loam soil.....	4.8	21.6	88	4.1
Do.....	7.5	22.0	143	6.5
Do.....	9.8	22.3	175	7.8
Do.....	12.2	22.3	161	7.2
Do.....	13.1	22.1	141	6.4
Marshall silt loam soil.....	5.4	18.7	102	5.5
Do.....	7.8	19.5	213	10.9
Do.....	10.2	20.2	351	17.4
Do.....	12.9	20.7	528	25.5
Do.....	14.8	21.1	671	31.8
Do.....	17.7	20.7	673	32.5
Do.....	20.3	19.8	471	23.8

The curves indicate a critical moisture content for the Sharkey clay soil of  $17\frac{1}{2}$  per cent, for the Marshall silt loam soil of  $15\frac{1}{2}$  per cent, and for the Norfolk fine sandy loam of 11 per cent. If greater accuracy than one-half of 1 per cent was desired, the briquettes were made up near the critical moisture content with increments of one-half of 1 per cent moisture and a new graph plotted. This method showed the critical moisture content for the Norfolk fine sandy loam soil, for example, to be at 11.3 per cent.

Briquettes of the Norfolk and Marshall soils were made up of the same samples of material used in the determination indicated in Table I to determine the effect of the amount of moisture on the breaking strength. These results are shown in Table II and graphically in figure 4. In this case, since the briquettes

were all of the same size, the weight of the briquette is taken as a measure of the density. It may readily be seen from figure 4 that the maximum load corresponds very closely to the maximum density of the briquette at the critical moisture content. Since the maximum load occurs at the critical moisture content, in comparing the breaking strength of two or more materials, each is made up at its critical moisture content.

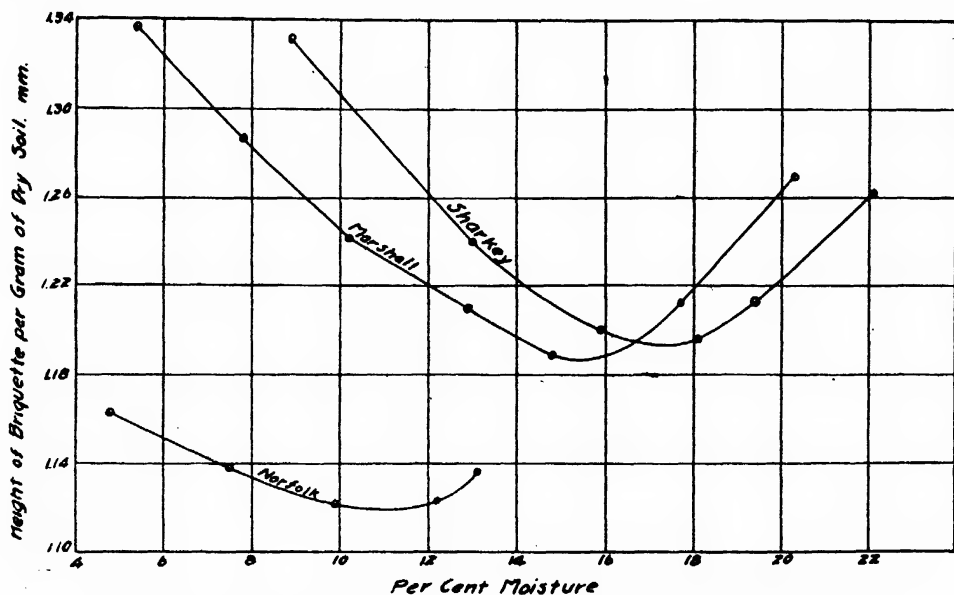


FIG. 3.—Showing the effect of the amount of moisture on the height of the briquette

#### TREATMENT OF THE MATERIAL

The treatment of the material before being made up into briquettes was found to be one of the most important factors as well as the most difficult to standardize. In order to get the water thoroughly distributed through the material a considerable amount of mixing was necessary, and in the matter of mixing the personal equation entered to a large extent. A mechanical method of mixing was tried out with but little success due to the fact that it was very difficult to control the amount of moisture in the mixture accurately, and the method could not be adapted to the small amounts of material available.

The method which was most easily duplicated in comparative tests of soils was to weigh out sufficient air-dry soil to make 100 gm. on the oven-dry basis. The soil was weighed out in a 250 cc. beaker and sufficient water added to bring it slightly above the critical moisture content. The material was then transferred to a glass mortar and rubbed gently with a glass pestle until the water was evenly distributed. It was then replaced in the beaker and allowed to stand in a desiccator containing distilled water for 42 hours. At the end of this time the material was weighed, the moisture content adjusted if necessary and the briquettes were made up as described above. This was designated as the first method.

The second method was the same as the first up to the point where the water was added. In this case the water was added gradually, the material being stirred with a spatula until sufficient water had been added to bring it to the consistency of thick cream. The material was then allowed to dry in the air with occasional stirring until it reached the critical moisture content. It was then transferred to the glass mortar and treated in the same manner as in the

first method. This method was found to give much higher results than the first method. This was probably due to the fact that the colloidal material was more thoroughly broken up and dispersed. However, the results were less uniform and very difficult to duplicate due to the fact that the same degree of dispersion was difficult to attain in two different samples of the same material. Another difficulty was encountered in drying the material. At the surface it would become very dry and hard while the interior was still very moist, so that by the time the critical moisture content of the whole sample was reached, the moisture was very unevenly distributed through the sample. This excessive local drying also caused the formation of aggregates in a manner that could not be duplicated.

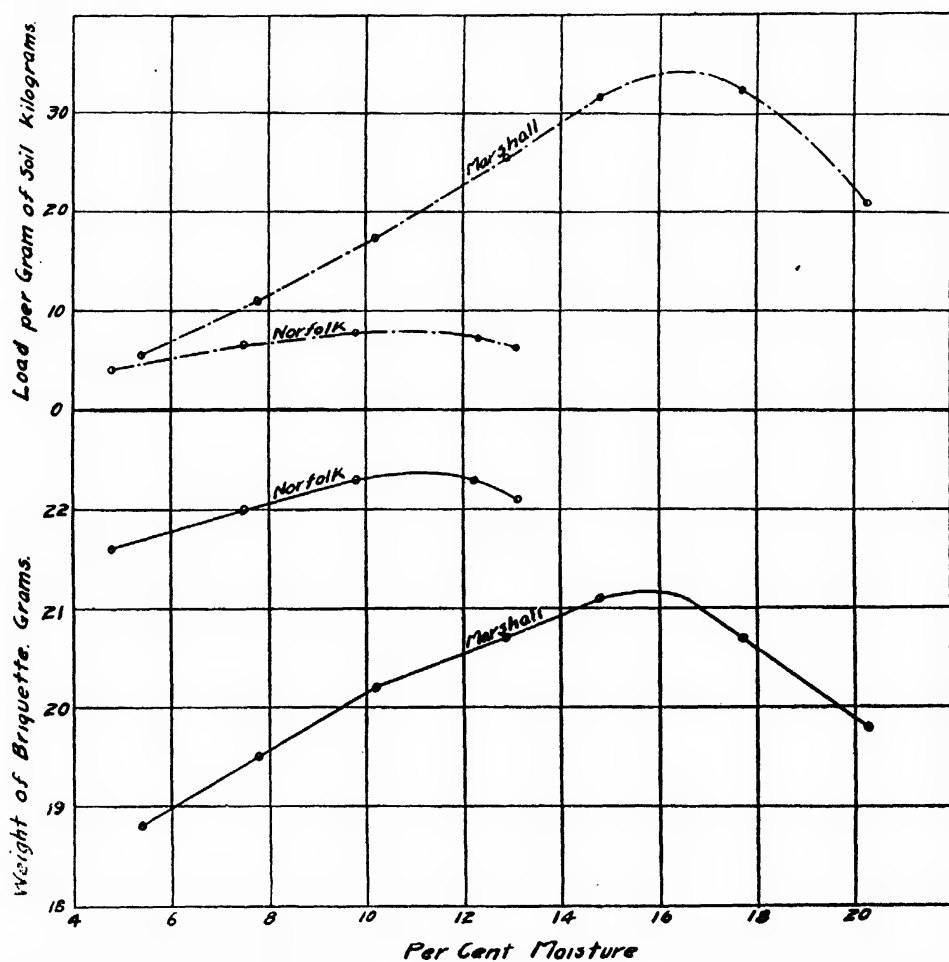


FIG. 4.—Showing the effect of the amount of moisture on the breaking strength of the briquette

In the third method, 100 gm. of soil was suspended in three liters of water, with every precaution being taken to get the maximum amount of deflocculation. The greater part of the excess water was drawn off through Pasteur-Chamberlain filters and the material dried down as described for the second method. This method consumed a large amount of time and on the two samples tried did not give results very greatly different from the second method. No attempt was made to duplicate the results by this method.

Table III shows the results obtained by the three different methods of treatment on two soils and by the first and second method on five soils. Since the first method could be duplicated with greater reliability in addition to requiring a shorter time to carry out, it was adopted as a standard.

TABLE III.—*Effect of method of treatment on the breaking strength of the briquette*

Soil	Colloid	Moisture	Load per gram of soil		
			First method	Second method	Third method
	<i>Per cent</i>	<i>Per cent</i>	<i>Kilograms</i>	<i>Kilograms</i>	<i>Kilograms</i>
Norfolk fine sandy loam soil.....	10.7	11.4	5.9	10.1	9.6
Marshall silt loam soil.....	27.3	16.0	30.7	37.5	39.8
Sharkey clay soil.....	61.2	18.0	75.2	103.8	-----
Ontario loam soil.....	18.5	14.0	12.8	19.6	-----
Ontario loam subsoil.....	11.4	12.5	16.2	31.1	-----

PRESSURE

The pressure used in making the briquettes could be controlled very easily by keeping the hydraulic pressure constant. The length of time which the pressure was applied to the material had some effect on the height of the briquette. Tests were made which indicated that the material came to equilibrium in from 30 to 60 seconds with a pressure of 2,000 pounds per square inch, so in making up the briquettes the pressure was applied to the material for 60 seconds. With the apparatus as described above it was difficult to get uniform briquettes at pressures less than 2,000 pounds per square inch. Tests were made with pressures up to 5,000 pounds per square inch, but as there was no apparent advantage in using more pressure than was necessary in securing uniform results, 2,000 pounds per square inch was adopted as standard. Under these conditions it was possible to make up four briquettes of equal quantities of a given material which would be within 0.05 mm. of the same height.

DRYING THE BRIQUETTES

Considerable difficulty was encountered in drying the briquettes so as to avoid cracking and checking. By removing the moisture very slowly and uniformly at the beginning of the drying this difficulty was largely overcome. It was found that after the moisture content had been reduced approximately 5 per cent the briquettes could be dried quite rapidly without danger of cracking. The method employed was to allow the briquettes to stand in a desiccator containing distilled water for 24 hours after being made up. At the end of this time they were transferred to an electrical drying oven, which was allowed to remain at room temperature and through which a very slow current of air nearly saturated with moisture was passed for 24 hours. Then the temperature of the oven was raised to 30° C. and the process continued 24 hours longer. The air was then shut off and the temperature of the oven raised to 110° C. for 18 hours. The briquettes were then transferred to a desiccator containing sulphuric acid and allowed to cool before being tested. Another method which gave equally as good results, but which took a little more time, was to have a series of four desiccators in which the saturation of air was, respectively, 100, 75, 50, and 25 per cent. The briquettes were allowed to stand 24 hours in each desiccator and then transferred to the drying oven at 110° C. The principal consideration in any method of drying is to so regulate the system that the moisture is supplied to the surface of the material to be dried at the same rate that it is carried off by evaporation so that the surface does not become dry and allow it to shrink and crack.

## THE BINDING POWER OF SOIL COLLOIDS IN THE SOIL

The binding power of the colloid in the soil was found to depend upon a number of factors. One important factor has been discussed by Gile et al. (4) in a previous publication, namely, the extractable and unextractable colloid. It would seem that the portion of the colloid which is the more easily dispersed would have a greater binding power than that which is more difficultly dispersed. There is no direct evidence that this is true, but it has been noted in the case of moist and air-dry colloids, which is more or less analogous. It has been noted in this laboratory that when a colloid in the moist condition is allowed to become air dry or oven dry, it is very difficult to redisperse. This is no doubt due to the formation of aggregates in the process of drying similar to the colloidal aggregates found in the soil.

Another factor of some importance is the difficulty of determining exactly the amount of colloid in the soil. The methods of determining the amount of colloid show considerable variation in some soils, but these variations are usually of small consequence in themselves. However, if the amount of colloid assumed to be in the soil is in error by a small per cent, when this is translated into the load per gram of colloid, the error will be magnified several times.

In determining the binding power of the soil colloids in the soil 17 soils in addition to the five listed in Table III were made up into briquettes by the first method, as described on page 500 and tested in the same way. These samples were subsamples of the same material used by Gile et al. (4) in determining the amount of colloid, and for this purpose the amount of colloid was assumed to be that shown by the water vapor adsorption. The results of these tests are shown in Table IV. If we assume that  $L$ , the load per gram of soil in kilograms, varies as  $C$ , the amount of colloid present, then

$$L = kC^n$$

TABLE IV.—The binding power of soil colloids in the soil

Soil	Colloid by water vapor ad- sorption	Weight of dry briquette	Average load per briquette	Load per gram of soil	Load per gram of colloid
	<i>Per cent</i>	<i>Grams</i>	<i>Kilograms</i>	<i>Kilograms</i>	<i>Kilograms</i>
Carrington loam soil.....	32.4	22.1	476	21.5	66.5
Carrington loam subsoil.....	29.4	25.2	497	19.7	67.1
Cecil clay loam soil.....	10.2	24.2	179	7.4	72.5
Cecil clay loam subsoil.....	31.5	23.8	410	17.2	54.7
Chester loam soil.....	8.5	22.7	231	10.2	119.6
Chester loam subsoil.....	24.8	23.5	485	20.6	83.3
Manor loam soil.....	18.4	23.2	404	17.4	94.6
Manor loam subsoil.....	18.6	23.3	399	17.1	92.2
Marshall silt loam soil.....	27.3	21.0	644	30.7	112.5
Marshall silt loam subsoil.....	34.3	22.9	1,188	51.9	151.4
Norfolk fine sandy loam soil.....	10.7	21.9	129	5.9	55.0
Norfolk fine sandy loam subsoil.....	20.5	24.4	431	17.7	86.1
Ontario loam soil.....	18.5	21.2	272	12.8	69.4
Ontario loam subsoil.....	11.4	23.3	378	16.2	142.2
Orangeburg fine sandy loam soil.....	6.2	22.0	45	2.0	33.0
Orangeburg fine sandy loam subsoil.....	21.5	25.1	361	14.4	66.9
Sassafras silt loam soil.....	8.0	22.0	127	5.8	72.2
Sassafras silt loam subsoil.....	18.9	23.0	447	19.4	102.8
Sharkey clay soil.....	61.2	21.1	1,585	75.2	122.8
Susquehanna clay subsoil.....	30.4	23.8	481	20.2	66.5
Wabash silt loam soil.....	29.8	21.9	1,006	46.0	154.2
Wabash silt loam subsoil.....	32.4	22.2	1,025	46.2	142.5

Substituting the values of  $L$  and  $C$  from Table IV in this equation and solving for the values of  $k$  and  $n$  which most nearly satisfy all of the equations

$$L=0.42\ C^{1.24}$$

This equation is shown graphically in figure 5 and also the observed values from Table IV.

If we let  $L_c$  represent the load per gram of colloid in kilograms, then by definition

$$L_c=\frac{L}{C}\times 100$$

Substituting in the above equation

$$L_c=\frac{0.42\ C^{1.24}}{C}\times 100=42\ C^{0.24}$$

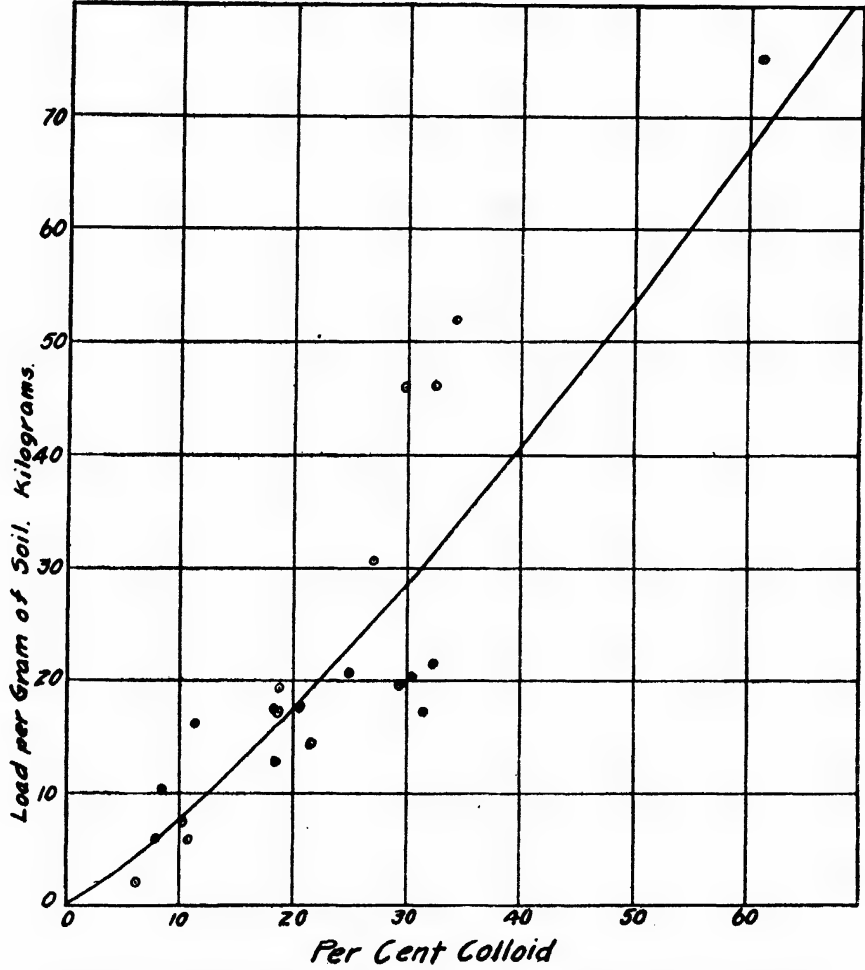


FIG. 5.—The relation between the load per gram of soil and the amount of colloid.

This indicates that under these conditions the load per gram of colloid depends upon the amount of colloid in the soil, thus

$$\begin{aligned} C &= 1, L_c = 42 \\ C &= 20, L_c = 86 \\ C &= 50, L_c = 107 \end{aligned}$$

Table IV shows this to be true in a general way, the soils with a small amount of colloid having a low load per gram of colloid and vice versa. However, the variations from the curve of the load per gram of soil as indicated in figure 4, indicate a much larger variation in the load per gram of colloid. Considering the

wide variation in the types and classes of soil, and the numerous factors affecting the binding power of the colloid, this relation seems to be very satisfactory. It is evident from the relation between the load and the amount of colloid that the amount of colloid in the soil could be calculated provided the load were known. This is somewhat analogous to the water adsorption factor as developed by Robinson (9). While this method is not adaptable to as great refinement and accuracy as the adsorption methods, it would seem to indicate the colloidal properties of soils for comparative purposes, particularly from the physical standpoint, more readily than the mere knowledge of the amount of colloid in each of the different soils under comparison. By combining the two methods, as was done in working out the above relation, one may be used as a control on the other. However, for the purpose of this discussion, it was assumed that the amount of colloid in the soil was that shown by the water vapor adsorption.

THE BINDING POWER OF SOIL COLLOIDS

In order to determine the binding power of soil colloids it was thought best to determine their breaking strength in controlled mixtures of sand. However, the binding power of the colloids was found to be influenced by a number of factors, as, for example, the size and grading of the sand particles with which they were mixed, the kind of colloid, that is, the soil from which the colloid was extracted, and to some extent by the amount of colloid present.

The particular colloid used in these determinations was extracted from the Sharkey clay soil by means of centrifuging and filtering as described by Moore et al. (7). The material was reduced to as low a moisture content as possible by means of the filters. It was then worked through a 1-millimeter sieve to make it uniform in consistency and placed in an air-tight jar to prevent evaporation. This material contained 30 per cent colloid (oven dry) and 70 per cent water. In mixing up a mixture of colloid and sand containing 25 per cent colloid, for example, it was necessary to mix 83.3 gm. of the above material with 75 gm. of sand. This made a total of 100 gm. of sand and colloid and 58.3 gm. of water. The materials were thoroughly mixed and then allowed to dry down to the critical moisture content, which had to be determined for each mixture. The briquettes were made up and treated in the same way as those of soil material.

The sand used in these experiments was separated from a clean, white river sand and from quartz flour. The silt and clay fractions were separated from the quartz flour by subsidence. The quartz flour contained a trace of colloidal material and the clay fraction was freed from this colloidal material by repeated centrifuging. Particular pains were taken to have all of the separations as exact as possible.

THE EFFECT OF SIZE AND GRADING OF THE MATERIAL

The first series of briquettes was made up of a mixture of 25 per cent colloid with each of the six grades of sand from coarse sand to clay. The results are shown in Table V and indicate that the binding power of the colloid increases as the size of the material decreases.

TABLE V.—Effect of the size of the material on the breaking strength of the briquettes, 25 per cent Sharkey colloid

Material	Size of particles	Weight of dry briquette	Average load per briquette	Load per gram of briquette	Load per gram of colloid
	Millimeter	Grams	Kilograms	Kilograms	Kilograms
Coarse sand.....	1.0 - .5	21.7	82	3.8	15.1
Medium sand.....	.5 - .25	21.4	91	4.3	17.0
Fine sand.....	.25 - .1	21.5	134	6.2	24.9
Very fine sand.....	.1 - .05	21.6	211	9.8	39.1
Silt.....	.05 - .005	21.4	526	24.6	98.4
Clay.....	.005 - .001	21.5	1,325	61.6	246.6

The next series of briquettes was made up of 25 per cent colloid with three different mixtures of sand of various grades. The results are shown in Table VI and indicate that the binding power of the colloid depends upon the grading as well as the size of the material. Mixtures *A* and *B* were prepared by mixing the indicated amounts of the relative sand sizes; mixture *C* was a quartz flour, the composition of which was determined by mechanical analysis.

TABLE VI.—*Effect of the grading of the noncolloidal material on the breaking strength of the briquettes, 25 per cent Sharkey colloid*

Name	Mixture of noncolloidal grains—mechanical analyses						Weight of dry briquette	Average load per briquette	Load per gram of briquette	Load per gram of colloid
	Coarse sand	Medium sand	Fine sand	Very fine sand	Silt	Clay				
A-----	30.0	25.0	20.0	15.0	10.0	0.0	Grams 24.9	Kilograms 526	Kilograms 21.1	Kilograms 84.6
B-----	10.0	15.0	20.0	25.0	30.0	.0	25.4	1,101	43.4	173.5
C-----	.0	.0	.3	13.3	71.0	15.4	23.8	1,690	71.0	284.0

It is natural to assume that the binding power is influenced by the amount of surface of the sand. The surface area of material of this character is very difficult to estimate accurately, but for purposes of comparison the particles were assumed to be cubes and the average diameter of the particles was assumed to be the longest diagonal of a cube. Then if  $D$  is the average diagonal measured in centimeters, and  $S$  is the total surface area of 1 gm. of material (specific gravity 2.65) measured in square centimeters

$$S = \frac{3.92^5}{D}$$

The surface of 1 gm. of each of the sands in Table V and the mixtures of sands in Table VI was calculated by this formula. The relation between the load and the surface was assumed to be of the form

$$L = kS^n$$

where  $L$  is the load per gram of briquette, and  $S$  is the surface area of 1 gm. of sand. For the sands separately the values of  $k$  and  $n$  which most nearly satisfied all of the equations were 0.42 and 0.52 respectively, while for the mixtures of sands they were 1.66 and 0.47 respectively. Substituting these values in the above equation, the loads were calculated for purposes of comparison with the observed loads. The results of these calculations are shown in Table VII and figure 6. It will be noted that the value of  $n$  is approximately the same in each case, the variation in strength under the two different conditions being indicated largely by the value of  $k$ .

<sup>5</sup> This equation is derived as follows:

The side of a cube in terms of the longest diagonal =  $\frac{D}{\sqrt{3}}$ .

The surface area of a cube in terms of the longest diagonal =  $2D^2$ .

The volume of a cube in terms of the longest diagonal =  $\frac{D^3}{3\sqrt{3}}$ .

The volume of 1 gram of sand =  $\frac{1}{2.65}$ .

The number of particles in 1 gram of sand =  $\frac{1}{\frac{2.65}{D^3}} = \frac{3\sqrt{3}}{2.65D^3}$ .

The total surface is equal to the number of particles multiplied by the surface area of 1 particle.

$$S = \frac{3\sqrt{3}}{2.65D^3} \times 2D^2 = \frac{3.92}{D}.$$



TABLE VII.—*Effect of surface area on breaking strength of the briquettes*

(a) CALCULATED FROM TABLE V

Material	Surface area of 1 gram	Load per gram of briquette	
		Observed	Calculated
	<i>Square centimeters</i>	<i>Kilograms</i>	<i>Kilograms</i>
Coarse sand.....	52	3.8	3.3
Medium sand.....	105	4.3	4.8
Fine sand.....	224	6.2	7.1
Very fine sand.....	523	9.8	11.0
Silt.....	1,425	24.6	18.7
Clay.....	15,685	61.6	65.4

$L=0.42 S^{0.52}$

(b) CALCULATED FROM TABLE VI

A.....	308	21.1	24.6
B.....	625	43.4	34.3
C.....	3,495	71.0	76.9

$L=1.66 S^{0.47}$

The above values are all based on a colloidal content of 25 per cent. Sufficient material was not available, particularly in the silt and clay groups, to test this relation extensively except in a general way. The value of *n* was found

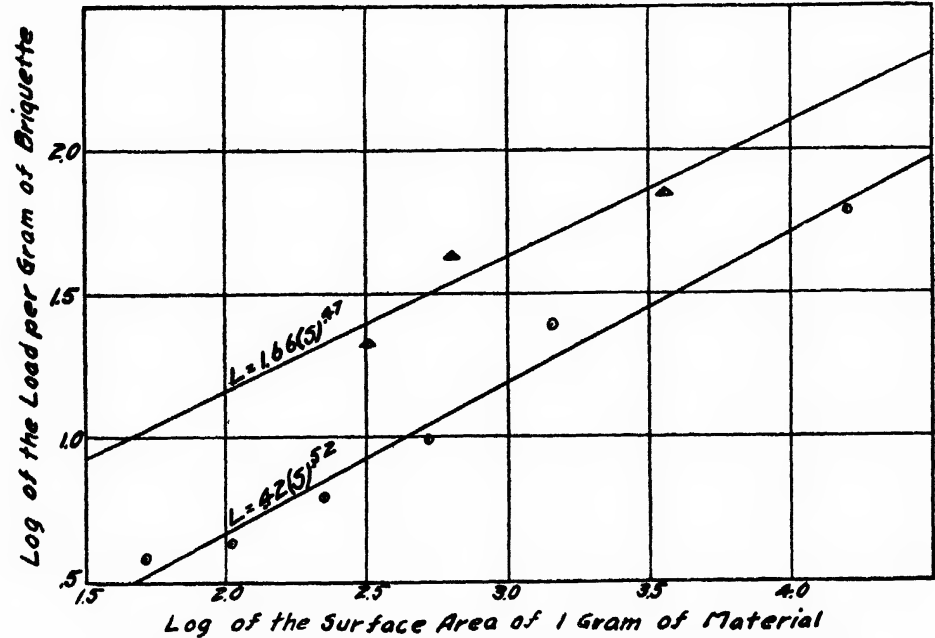


FIG. 6.—Showing the effect of the surface on the breaking strength of briquettes

to be practically constant as shown above, the variation in strength due to size and grading of the material and the amount of colloid present being indicated by variation in the value of *k*.

THE EFFECT OF THE AMOUNT OF COLLOID

To determine the effect of the amount of colloid present in a mixture on the binding power of the colloid, varying amounts of colloid were mixed with quartz flour and also with medium sand. The results of these tests are shown in Table VIII. It may be noted from the methods of calculating the load per gram of

colloid, that if the load per gram of briquette varied directly as the amount of colloid present, the load per gram of colloid would be constant for each material. With quartz flour this is practically the case with the exception of the first determination (50 per cent colloid). With medium sand the variation is larger. It may also be noted that the variation in the load per gram of colloid, particularly with medium sand, follows more or less closely the density of the material as indicated by the weight of the briquette.

TABLE VIII.—*Showing the effect of the amount of colloid on the breaking strength of briquettes*

Sharkey colloid and—	Per cent of colloid	Weight of briquette	Average load per briquette	Load per gram of briquette	Load per gram of colloid
		<i>Grams</i>	<i>Kilograms</i>	<i>Kilograms</i>	<i>Kilograms</i>
Quartz flour.....	50.0	21.4	1,645	76.9	153.8
Do.....	25.0	23.8	1,690	71.0	284.0
Do.....	10.0	21.2	590	27.8	278.3
Do.....	3.6	19.9	218	10.9	303.9
Medium sand.....	50.0	21.3	322	15.1	30.2
Do.....	40.0	21.9	281	12.8	32.1
Do.....	30.0	21.5	209	9.7	32.4
Do.....	20.0	20.4	100	4.9	24.5
Do.....	10.0	18.1	43	2.4	23.8

#### THE BINDING POWER OF DIFFERENT SOIL COLLOIDS

There were a few soil colloids available in the laboratory in sufficient quantity to make a few briquettes. These colloids were in the air-dry condition while the Sharkey colloid used in the above experiments was in the moist condition. The results of these tests are shown in Table IX. The five different colloids studied show rather wide variations in binding power. The different soil colloids show rather wide differences in adsorptive power, as well as in general character and appearance, so it was but reasonable to assume that there would be differences in binding power; however, the differences were not expected to be as great as these results indicate. Perhaps when these materials are available in larger quantities and in the moist condition, a more adequate explanation of the differences in binding power may be given.

TABLE IX.—*Showing the effect of the kind of colloid on the breaking strength of briquettes*

(a) 25 PER CENT COLLOID (AIR-DRY) AND QUARTZ FLOUR

Colloid from—	Weight of briquette	Average load per briquette	Load per gram of briquette	Load per gram of colloid
	<i>Grams</i>	<i>Kilograms</i>	<i>Kilograms</i>	<i>Kilograms</i>
Stockton clay adobe soil.....	24.0	1,726	72.0	288.0
Sharkey clay soil.....	21.9	1,015	46.4	185.5
Cecil clay loam subsoil.....	23.3	664	28.5	114.0

(b) 10 PER CENT COLLOID (AIR-DRY) AND FINE SAND

Norfolk fine sandy loam soil.....	18.8	61.2	3.3	32.5
Marshall silt loam soil.....	19.0	90.7	4.8	47.8
Sharkey clay soil.....	18.9	108.8	5.8	57.6

## GENERAL DISCUSSION

Some of the factors influencing the binding power of soil colloids have been outlined above. It was first necessary to establish a suitable method for determining the binding power and to ascertain the conditions under which the results from this method could be relied upon. The method of determining the breaking strength of briquettes was adopted as most applicable, and when proper care was taken in making them, the results could be duplicated with sufficient accuracy.

In order to obtain duplicable and therefore comparable results the briquettes must be made up with (a) the critical amount of moisture present, that is, the moisture content when the briquettes with 2,000 pounds pressure applied assumed the smallest volume, (b) the same degree of dispersion of the colloid and a uniform distribution, (c) the same pressure per square inch (2,000 pounds) for the same length of time and (d) drying in such a manner as to prevent cracking of the briquettes. A variation of any of these factors might so affect the breaking strength of the briquettes that the values obtained would be very much in error. These four factors, which are involved in the formation of the briquettes and influence the values obtained, were standardized as nearly as possible. Working under the standardized conditions, the effect of the following factors on the binding power of the colloid was studied: (a) The amount of colloid present, (b) the kind of colloid, (c) the size of the mineral grains, (d) the grading of the sizes of the mineral grains, and (e) the surface area of the mineral grains. These factors are variable and interdependent so that great accuracy is not obtainable in determining them.

Tests on soils showed a general relation expressed in the formula  $L = .42C^{1.24}$ . An additional factor is involved, in the case of soil, in the determination of the amount of colloid in the soil. The variation in load is in direct proportion to the amount of colloid present, the constants in the equation changing with the character of the colloid and the mineral portion of the soil.

Tests on mixtures of colloid and sand show that with decreasing size of sand grains there is an increasing value for the binding power of the colloid. The increase in binding power is not directly proportional to the decrease in the average diameter of the sand grains. For example, in Table V the medium sand with 25 per cent colloid gives a load per gram of colloid of 17 kgm., but when very fine sand is used with a mean diameter one-fifth that of medium sand, the binding power is approximately twice as great. However, with the silt and clay sizes the increase in binding power is larger in proportion than the decrease in diameters.

This indicates that the amount of surface has a very great influence. The calculation of surfaces as shown in Table VII shows that it is probable that a relation does exist, though the data for a complete verification of the relation are not available.

The kind of colloid is of great importance in determining the binding power of mixtures. Of those colloids tested, that from the Stockton clay adobe soil had the greatest binding power and that from the Cecil clay loam subsoil the least. The reason for this difference has not been determined, but it may be due to a difference in the chemical composition, in the size or aggregation of the colloidal particles, or to other factors.

This discussion gives some idea of the nature of the problem of determining the binding power of colloidal material in the soil. The complexity of the problem is pointed out and the influence of the various factors is recognized. While the factors and their influences have been partially determined, it yet remains to coordinate and fully evaluate them, so that more exact information of the character of a soil may be derived from the determination of the colloid content and one or more of these factors.

## SUMMARY

1. A method is outlined for testing the binding power of soil colloids by determining the breaking strength of briquettes molded from soils under definitely established conditions.

2. It is shown that the factors influencing the breaking strength of a briquette are:

- (a) The amount of moisture present at the time of molding.
- (b) The treatment of the material before molding the briquette.
- (c) The pressure applied.
- (d) The manner of drying.

3. Some of the factors found to affect the binding power of soil colloids are:

- (a) The amount of colloid present.
- (b) The size and grading of the noncolloidal material.
- (c) The kind of colloid.
- (d) The dispersion of the colloid.

4. A general relation between the load per gram of soil  $L$ , and the amount of colloid in the soil in per cent  $C$ , has been deduced and given expression in the formula,  $L = .42 C^{1.24}$ .

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# THE GENETIC RELATION BETWEEN TRITICUM DICOCCUM DICOCCOIDES AND A SIMILAR MORPHOLOGICAL TYPE PRODUCED SYNTHETICALLY<sup>1</sup>

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The synthetic production of a form similar to the wild emmer (*Triticum dicoccum dicoccoides* Kcke.) has been reported by the authors of this paper.<sup>2</sup> This synthetic form resulted from a cross between Early Red Chief, a variety of *Triticum vulgare* Vill., and one the writers received under the name Marouani, a variety of *Triticum durum* Desf. It is possible that the name Marouani was a misnomer, as Dr. C. R. Ball, on examining the sample, stated that it more nearly resembled the variety Peliss.

In the earlier paper it was noted that the chief difference between the true wild emmer and the synthetic form is the width of spikelets. The kernels of the synthetic type are broader than those of the true wild form as illustrated by the samples on hand. This causes the spikelets to be broader. The true wild emmer, however, is very variable and some forms are found that have very broad spikelets, even resembling the synthetic form.

One of the chief characteristics of the wild emmer is the fragility of the rachis. The articulation is such that at maturity the spikelets separate one from another very readily. The rachis segment which bears a spikelet remains attached to the base of the spikelet when the spike disarticulates, just as in ordinary emmer. Disarticulation occurs so easily that it is very difficult to obtain a head of the wild emmer intact. The synthetic form, Pl. 1, B, is very similar to this wild one, Pl. 1, A.

As the synthetic form, which was produced as a result of the cross, so nearly resembled the true wild emmer in all its visible characters, it seemed worth while to compare the two forms as to their genetic behavior. With this in mind the two have been crossed upon the same variety.

Two different kinds of crosses were made for this study. In one case a durum wheat, the Kubanka, was crossed with the two wild types, and in the other case both of them were crossed with Black Winter emmer. In the discussion of these results the synthetic type will be referred to as synthetic wild and the true wild emmer as true wild or simply as wild.

## THE F<sub>1</sub> GENERATION

The F<sub>1</sub> plants resulting from the two crosses, where the true wild and synthetic wild were crossed with Kubanka, prove to be quite similar. The culms are solid or full of pith below the head. The spikes break up readily, indicating the domi-

<sup>1</sup> Received for publication March 29, 1924. Published as Paper No. 129, Department of Plant Breeding, Cornell University, Ithaca, New York.

<sup>2</sup> LOVE, H. H., and CRAIG, W. T. THE SYNTHETIC PRODUCTION OF WILD WHEAT FORMS. Jour. Heredity 10: 51-64, illus. 1919.

nance of the fragile rachis. On first examination it seemed that those heads coming from the cross in which the true wild was used were more fragile, but after thorough drying there seemed to be no difference. The  $F_1$  heads in both crosses have a flattened appearance similar to those of the wild type and the rachis is heavily pubescent, as is the case in the wild forms. The awns resemble those of the wild parents and the kernels have the red color of the wild forms. In shape the kernels are longer than those of Kubanka, but are somewhat broader than those of the wild forms.

The  $F_1$  plants which resulted from crossing the two wild types with Black Winter emmer were alike in each case. So far as the characters mentioned above are concerned, these  $F_1$  plants were similar to those from the Kubanka crosses with the exception that the glumes of the hybrids with Black Winter emmer were black or grayish-black. The color is slightly lighter than that of the Black Winter emmer.

### THE $F_2$ GENERATION

When the second generation was grown there was considerable diversity of form and of degree of fragility of the rachis. Some of the characters will be discussed separately.

The first character to be considered is the fragility of the rachis. Owing to the nature of this character, it is very difficult to classify. This is especially true if the material has not dried thoroughly. Some of the rachises are brittle only toward the tip of the head while others may be fragile the entire length of the head, except for a few spikelets at the base. In the wild emmer usually two or three spikelets at the base of the head are held rather firmly.

Various tests were used in order to classify this material, such as natural shattering, readiness of breaking when the spike was shaken, or when the spikes were subjected to some stress, and so on. Finally, the classification was made by means of the above tests plus an examination of the end of the rachis segment under a lens. The rachis of the true wild emmer disarticulates in such a way that the end of the rachis segment is round and perfectly smooth. This test was then applied and all plants that showed disarticulation leaving the rachis segments with smooth round ends were classed as fragile. If the end of the rachis segment was broken or torn the plants were classed as tenacious. The results of segregation for this character in the second generation plants are given in Table I.

TABLE I.—Segregation of the second generation of crosses of *Kubanka durum* wheat and Black Winter emmer with the wild and the synthetic wild emmer for character of rachis, shown by manner of disarticulation

	Number of plants having—				Devi- ation	Prob- able error	Devia- tion divided by probable error
Cross	Fragile rachis		Tenacious rachis				
	Observed	Calcu- lated	Observed	Calcu- lated			
Kubanka X true wild.....	134	133.5	44	44.5	0.50	3.90	0.13
Kubanka X synthetic wild.....	147	150.0	53	50.0	3.00	4.13	0.73
Black Winter emmer X true wild.....	216	219.38	18	14.62	3.38	2.50	1.35
Black Winter emmer X synthetic wild.....	221	227.8	22	15.20	6.80	2.55	2.67

From these results it is very evident that, so far as this character is concerned, the segregation for fragile and for tenacious rachis gives about the same ratio whether the wild or synthetic type is used as the parent. It seems evident that

in the crosses with Kubanka, the segregation follows the simple 3:1 ratio. The deviation from the expected numbers is very small compared with the probable errors. This ratio was established further by an examination of some  $F_3$  material.

In the case of the crosses with Black Winter emmer a different condition exists. Here the segregation suggests a 15:1 ratio. The deviation from the expected numbers is within the limit of three times the probable error in each case. Such behavior may be expected from emmer as it has a different method of disarticulation from that of Kubanka. The emmer spike does not break up readily but when the rachis disarticulates the spikelet carries the rachis segment which bore it. This fact would lead one to expect a behavior in inheritance different from that in the case of the Kubanka wheat.

These results show very plainly that, so far as these two crosses are concerned, the true wild and the synthetic wild are very similar in their behavior with respect to the inheritance of the character of the rachis.

The different types of heads obtained from these crosses are shown in Plates 2 to 8, inclusive.

In Plate 2 are shown two heads resembling those of Black Winter emmer. Head A was obtained from crossing Black Winter emmer with the true wild, and head B from crossing it with the synthetic wild.

The heads shown in Plate 3 also are from the crosses with Black Winter emmer. Heads A and C are from the cross with the true wild and heads B and D from that with the synthetic wild. These illustrations show the great similarity between the heads from the two different crosses. Heads A and B have a tenacious rachis and heads C and D a fragile rachis. These heads all have black glumes.

The heads in Plate 4 also are from the crosses between Black Winter emmer and the two wild emmers. These heads have brown glumes and are selected to show the tenacious and the fragile rachis from the two crosses. Heads A and C are from the cross in which the wild was used as a parent and heads B and D are from the cross where the synthetic wild was used.

The heads in Plate 5 also are from the crosses between Black Winter emmer and the two wild forms. Heads A and B are from the cross with the true wild and heads C and D from the other cross. All have white glumes. They again show the close resemblance between the heads from the two crosses.

The heads from the crosses between Kubanka durum and the two types of wild emmer are shown in Plates 6, 7, and 8. Heads A and C of each plate are from the cross with the true wild and heads B and D of each plate are from the cross with the synthetic wild. The heads in Plate 6 resemble those of emmer to some extent. The heads in Plate 7 are more like those of durum, and the heads in Plate 8 are similar to those of spelt except that the articulation is more like that of emmer. In general, it is clear that heads obtained from the two types of crosses are very similar and indicate that the synthetic wild carries the same factors for head type as does the true wild.

These crosses did not show any segregation for color of kernel in the series where Black Winter emmer was used. This indicated that the constitution of the two types of wild was the same as that of Black Winter emmer. In the crosses where Kubanka was used, however, segregation did occur. (See Table II.)



TABLE II.—*Segregation in  $F_2$  for color of kernel in crosses between Kubanka durum and the two wild emmers*

Cross	Numbers of plants and kernel color				Devia- tion	Probable error	Devia- tion divided by proba- ble error.
	Observed		Expected				
	Red	White	Red	White			
Kubanka X true wild .....	164	14	166.9	11.1	2.9	2.18	1.33
Kubanka X synthetic wild .....	185	15	187.5	12.5	2.5	2.31	1.08

TABLE III.—*Segregation in  $F_2$  for color of glumes in crosses between Black Winter emmer and the two wild emmers*

Cross	Numbers of plants having color of glumes			
		Black	Brown	White
Black Winter emmer X true wild .....	Observed	181	43	10
	Expected	175.2	43.8	14.6
Black Winter emmer X synthetic wild .....	Observed	173	53	17
	Expected	182.4	45.6	15.2

These results indicate a 15:1 ratio and the deviations are not very large. In fact, when they are compared with their probable errors they are not significantly different from what one would expect. The red color of the true wild and the synthetic wild must, therefore, depend upon two factors.

From another cross where the true wild was used a segregation of 15 red:1 white was obtained. It is rather interesting that this synthetic wild emmer should have the same genetic constitution for color of kernel as the true wild.

The crosses between Kubanka and the wild emmers did not show any segregation for color of glumes, as both parental forms had white or yellowish-white glumes. In the case of the Black Winter emmer crosses, however, there was segregation, as would be expected, with contrasted glume colors. (See Table III.)

The results show that the segregation follows a 12:3:1 ratio. That is, the Black Winter emmer carries both the factor for black or purple glume color and the one for brown color. While the observed and expected numbers show some deviation, they are in fair agreement, considering the numbers of plants. For the cross where the true wild emmer was used  $P$  equals 0.450, and for the one where the synthetic wild was used  $P$  equals 0.392.

The distribution and closeness of fit show very clearly that the two types of wild emmer react in a very similar way when crossed upon the Black Winter emmer. This fact is further emphasized when the pubescence of the glume is considered. The pubescence of the glume of the Black Winter emmer is linked with the black or purple color of the glume. The cross with the true wild gives 181 black pubescent and 53 nonblack glabrous, while the cross with the synthetic wild gives 173 black pubescent and 70 nonblack glabrous. These numbers deviate somewhat from the expected 3:1 ratio, yet if the numbers of plants were larger no doubt the agreement would be closer. Regarding other characters, such as the number of spelt or emmer types occurring in the different crosses, the data show that the results are about the same no matter whether the true wild or synthetic wild was used as a parent.

So far as these studies have been conducted it is evident that the synthetic wild emmer, which occurred as a result of crossing a variety of *Triticum vulgare* and a variety of *Triticum durum*, is the same as, or certainly genetically very similar to, the true wild emmer found in Palestine.<sup>3</sup> The earlier paper mentioned above<sup>4</sup> shows that, so far as the visible characters are concerned, the two forms are alike, and it is a very interesting fact that genetically the same is true. These two forms are now being compared in other ways. The evidence seems to indicate that the synthetic form will repeat in every way the behavior of the true wild form.

Such evidence, however, does not yet answer the question as to whether this wild emmer, *Triticum dicoccum dicoccoides*, is the progenitor of all other types. Argument may be presented that it is the progenitor or that it is only a contemporary form. One fact has been established, namely, that through hybridization a form similar morphologically and genetically to the true wild type has been found. That this is no mere accident is supported by the fact that from still other crosses other synthetic forms of wild emmer have been obtained.

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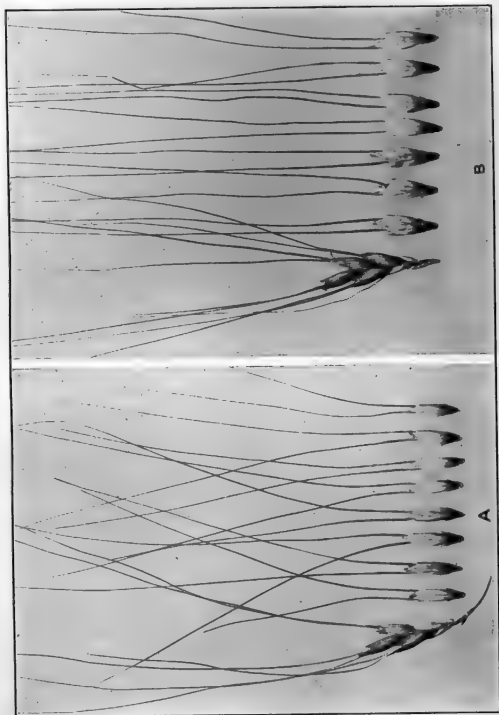
<sup>4</sup> LOVE, H. H., and CRAIG, W. T. THE SYNTHETIC PRODUCTION OF WILD WHEAT FORMS. Jour. Heredity 10: 51-64, illus. 1919.

**PLATE 1**

Basal portion of spike and individual spikelets of the true wild (A) and synthetic wild (B) emmers.

(520)

520a



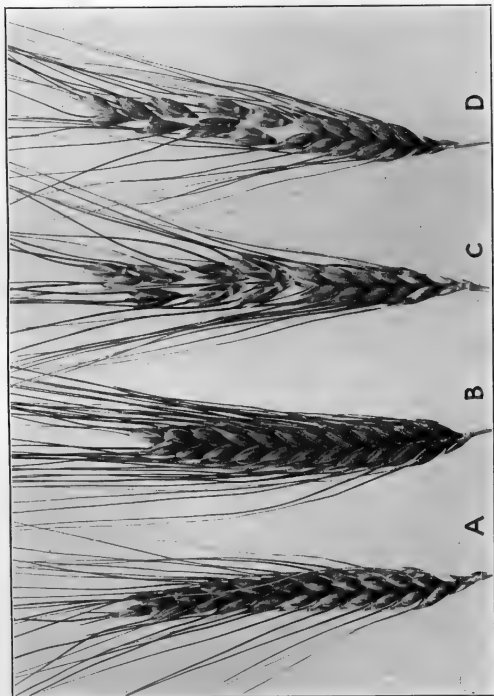


## PLATE 2

Spikes of the F<sub>1</sub> generation, resembling those of Black Winter emmer, resulting from crossing Black Winter emmer with the true wild and synthetic wild emmers.

### PLATE 3

Black-glumed spikes of the  $F_2$  of the crosses between Black Winter emmer and the two wild emmers. Heads A and C from the cross between the true wild and heads B and D from the cross with the synthetic wild.





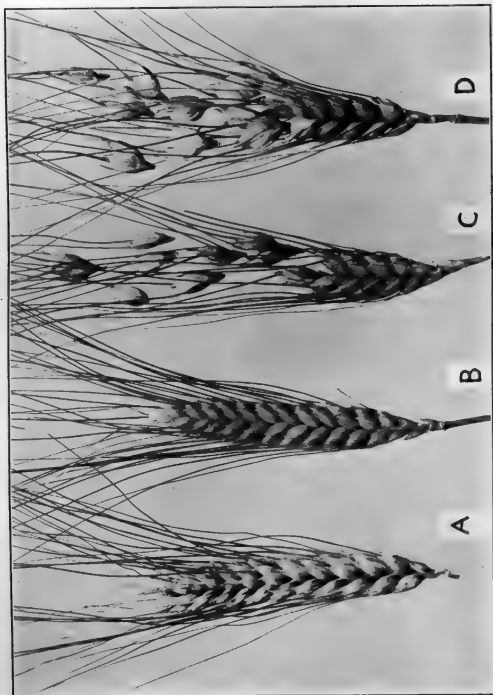


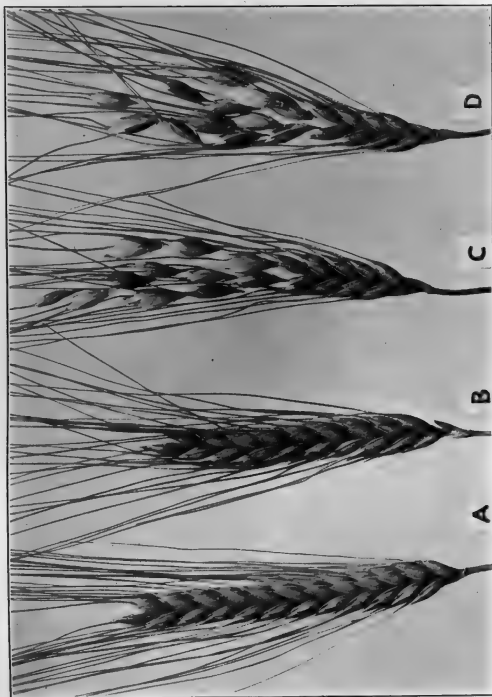
#### PLATE 4

Brown-glumed spikes of the  $F_2$  of the crosses between Black Winter emmer and the two wild emmers. Heads A and C from the cross between the true wild and heads B and D from the cross with the synthetic wild.

### PLATE 5

White-glumed spikes of the  $F_2$  of the crosses between Black Winter emmer and the two wild emmers. Heads A and C from the cross between the true wild and heads B and D from the cross with the synthetic wild.





## PLATE 6

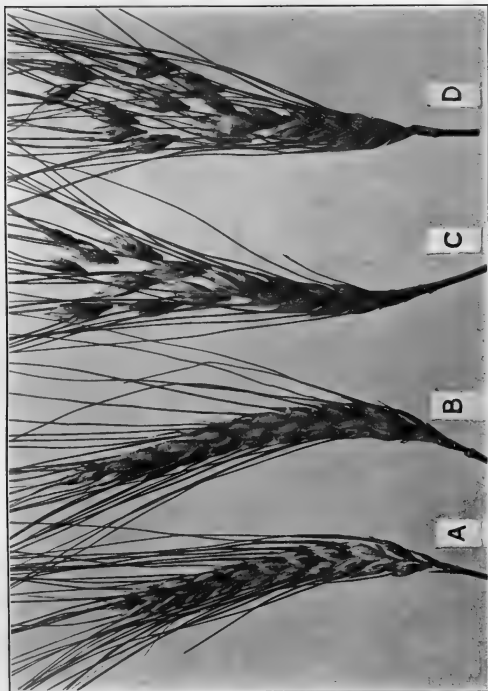
Spikes, somewhat resembling those of emmer, from the F<sub>2</sub> generation of the cross between Kubanka durum and the two kinds of wild emmer. Heads A and C from the cross between the true wild and heads B and D from the cross with the synthetic wild.

## PLATE 7

White-glumed spikes, resembling those of durum wheat, from the cross between Kubanka durum and the two kinds of wild emmer. Heads A and C from the cross between the true wild and heads B and D from the cross with the synthetic wild.







## PLATE 8

Spelt-like spikes of the  $F_2$  from the cross between Kubanka durum and the two kinds of wild emmer. Heads A and C from the cross between the true wild and heads B and D from the cross with the synthetic wild.

# BUD SELECTION AS RELATED TO QUALITY OF CROP IN THE WASHINGTON NAVAL ORANGE<sup>1</sup>

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## INTRODUCTION

The occurrence of bud variations in citrus trees and the relation of some of them to the origin and development of a number of strikingly diverse strains of the commercial citrus varieties grown in California have been presented in earlier publications.<sup>2</sup> Evidence that quantity of production may be perpetuated through bud propagation of limb variations of the Washington Naval orange has recently been published.<sup>3</sup> Many marked differences in quality of fruit, apparently resulting from bud variation, have been under observation and several are now being studied in progeny plantings. A variation of fruit quality, directly traceable to bud variations in trees of both the Washington and Thomson strains of the Washington Naval orange, is herein described.

## FACTORS DETERMINING THE QUALITY OF CROP

The term "quality of crop" is here used to define the market grade of the fruit as affected by its physical characteristics. The size, color, shape, texture, and blemishes of the fruit determine its commercial grade in large measure. The thickness of the rind, amount and flavor of the juice, character of the rag, and the number of seeds are also important physical characteristics of the fruits from the commercial standpoint. Fruits of medium size for the variety are usually the most valuable, being most desired by the consumer and most profitable for the producer. A reddish-orange color is most desirable, while a pale or yellowish color usually lowers the merchandising value of the fruit. In connection with their packing and marketing, uniformity of shape within the variety is an important character. Corrugated, rough fruits or those having an uneven surface are usually assorted into lower grades, a skin of uniformly smooth texture being most desirable. Blemishes most commonly found are scars, scratches, insect injuries, diseased areas, and spots which are apparently due to physiological weaknesses of the rinds.

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<sup>1</sup> Received for publication January 22, 1924.

<sup>2</sup> The following department bulletins summarize the results of the studies of bud variations in the Washington Naval and Valencia orange, Marsh grapefruit, and Eureka and Lisbon lemon varieties, respectively:

SHAMEL, A. D., SCOTT, L. B., and POMEROY, C. S. CITRUS-FRUIT IMPROVEMENT: A STUDY OF BUD VARIATION IN THE WASHINGTON NAVAL ORANGE. U. S. Dept. Agr. Bul. 623, 146 p., illus. 1918.

———— CITRUS-FRUIT IMPROVEMENT: A STUDY OF BUD VARIATION IN THE VALENCIA ORANGE. U. S. Dept. Agr. Bul. 624, 120 p., illus. 1918.

———— CITRUS-FRUIT IMPROVEMENT: A STUDY OF BUD VARIATION IN THE MARSH GRAPEFRUIT. U. S. Dept. Agr. Bul. 697, 112 p., illus. 1918.

———— CITRUS-FRUIT IMPROVEMENT: A STUDY OF BUD VARIATION IN THE EUREKA LEMON. U. S. Dept. Agr. Bul. 813, 88 p., illus. 1920.

———— CITRUS-FRUIT IMPROVEMENT: A STUDY OF BUD VARIATION IN THE LISBON LEMON. U. S. Dept. Agr. Bul. 815, 70 p., illus. 1920.

<sup>3</sup> SHAMEL, A. D., POMEROY, C. S., and CARYL, R. E. BUD SELECTION AS RELATED TO QUANTITY PRODUCTION IN THE WASHINGTON NAVAL ORANGE. Jour. Agr. Research 26: 319-322, illus. 1923.

## PROGENY TESTS OF BUD VARIATIONS

The individual-tree performance-record studies of many trees possessing marked limb variations which have been described in earlier reports suggest the probability that the established trees of diverse strains occurring in the performance-record plats and similar ones observed in commercial orchards were unintentionally propagated from limb variations.

In order to secure definite evidence upon the perpetuation of diverse strains with especial reference to quality of fruit, experimental propagations were begun in 1915 using bud wood secured from limb variations which resembled in fruit and foliage characteristics some of the trees of several of the strains under investigation. These propagations were made on selected sour orange seedlings in a commercial nursery in cooperation with the Citrus Experiment Station of the University of California and some of the resulting progenies were planted on the grounds of the Citrus Experiment Station at Riverside, Calif., in July, 1917. The land on which these progeny plantings are located had previously been planted only to winter grain crops which had been grown without irrigation or fertilization. Little or no fertilizer has been used in this experimental progeny orchard other than that secured from the legume cover crop growth which has been plowed under once or twice each year and no pruning of the trees has been done thus far.

Each progeny was planted so that the trees stand 10 feet apart in the same row, this close planting providing for many more trees in the available area than could otherwise have been grown. The usual cultivation and irrigation methods have been practiced and orchard heaters have been provided for frost protection during periods of low temperatures. The performance record of each tree has been secured every year since they came into bearing, particular attention being given to recording the number and quality of variants from the normal type of fruit.

## BROWN-SPOTTED AND EARLY RIPENING LIMB VARIATIONS

The parent tree in which the first limb variation of this type was discovered is a tree of the Thomson strain of the Washington Navel orange variety on the Victoria ranch of the National Orange Co., at Riverside, Calif., which came to the attention of the senior writers in 1915 when it was 12 years old. The root-stock used in the propagation of the parent tree was sweet seedling orange. The size of the tree and the appearance of the foliage indicated a normal and healthy condition of growth.

The fruits borne by the parent tree are quite variable, the majority being classed as typical of the Thomson strain while some of them very closely resemble typical fruits of the Washington strain. The Thomson fruits are distinguished by very smooth and comparatively thin rinds having a bright reddish-orange color. The flesh is coarse and the fruits usually lack juice to such a degree as to render them very much less desirable than are those of the Washington strain. The fruits of the Washington strain have somewhat coarser and thicker orange-colored rinds but have very tender flesh with an abundance of juice, making them very desirable.

One limb of this parent tree produces very early ripening fruits of small size, distinctly flattened, pale yellow in color with occasional reddish stripes and marked with sunken brown spots of irregular shapes and sizes. The spotted oranges usually have only a small or rudimentary navel and frequently the navel opening is entirely closed. The rinds of the fruits are usually very thick and the flesh coarse and tough. The fruits ordinarily have but little juice which is normally lacking in both acid and sugar, making them very undesirable. The fruits mature and most of them drop from the tree during November and De-

cember and after the oranges drop the brown spots increase in number and size. Plate 1, A, shows typical fruits from this limb variation and Plate 1, B, shows typical Thomson fruits from a normal limb on the same tree. It was first thought that these spots were due to fumigation burns, and when this theory was proven to be incorrect it was suggested that they might have been caused by hail injuries. The fact that the fruits on adjacent trees and those on the parent tree other than the ones borne by the limb variation were free from these spots indicated that they were not caused by hail. This conclusion has been confirmed by the fact that in each successive season the fruits on the limb variation have shown similar spots, while the fruits on the other branches have remained normal in this respect, and during several of these seasons no hail fell in the vicinity.

It was thought for a time that the spotted condition of the fruit might be due to a fungus disease, but careful pathological studies by two independent investigators failed to reveal any evidence of disease. Subsequent observations and studies of the spotted fruits have led to the conclusion that the tissue of the rinds of the fruits is inherently weak and breaks down rather easily as the oranges approach maturity. The spots have a similar appearance to those which develop on citrus fruits where the oil cells have been broken and the free oil becomes spread over the adjacent areas of the rinds.

The parent tree on which the second limb variation of this kind was found is a tree of the Washington strain of the Washington Navel orange variety, which had been propagated on sweet-seedling orange rootstock and planted in 1912. This parent tree is located in the orchard of the Lemona Heights ranch, which is about 3 miles distant from the Victoria orchard where the other tree showing this type of variation was found.

The limb variation in the tree of the Washington strain was found to produce fruits which were very similar to those borne by the limb variation in the tree of the Thomson strain described. The fruits are smaller than those borne by the normal branches of the tree, more flattened in shape, and have a yellowish color with occasional red stripes or sections. The rinds are thicker and coarser in texture than is the case with the normal fruits on this tree. The fruits mature much earlier than those on the normal branches and drop easily, most of them falling to the ground during the months of November and December. The fruits develop small, irregular sunken brown spots on the surface of the rinds very similar in appearance to those found on the limb variation described above. None of these brown spots has been found on the reddish-colored sections which occur on some of the fruits.

### PROGENY PROPAGATION

Buds secured from each of the limb variations of the two parent trees described above were propagated on sour orange seedlings in the spring of 1915. The resulting trees were planted on July 3, 1917, in the orchard of the Citrus Experiment Station of the University of California, only two of each progeny being planted owing to the limited available space. In the case of the Thomson strain variation, the progeny test consists of two trees grown from buds secured from the spotted-fruit limb variation and two trees grown from buds taken from a normal Thomson limb.

The progeny trees have made a healthy and normal growth and came into production during the 1920-21 season when they were 3 years old. All four of the progeny trees from the two spotted-fruit limb variations are smaller in size and have a more compact habit of growth than normal or typical trees of the strains. Plate 2 shows the comparative size and habit of growth of a progeny tree propagated from the spotted-fruit limb variation in the parent Thomson tree and a progeny tree from the normal portion of the same parent tree.

## PROGENY PERFORMANCE RECORDS

The performance records of the progeny trees of the two spotted-fruit limb variations of the Thomson and Washington strains and of the normal part of the parent tree of the Thomson strain are shown in Tables I, II, and III.

TABLE I.—*Number of oranges produced by the two progeny trees which were propagated from the spotted-fruit limb variation in a tree of the Thomson strain*

Tree No.	1920-21	1921-22	1922-23	1923-24	Total
1.....	0	1	59	155	215
2.....	25	8	92	282	407
Total.....	25	9	151	437	622

TABLE II.—*Number of oranges produced by the comparative progeny trees propagated from a normal limb of the parent tree of the Thomson strain*

Tree No.	1920-21	1921-22	1922-23	1923-24	Total
1.....	10	95	92	242	439
2.....	7	107	96	370	580
Total.....	17	202	188	612	1,019

It will be noted that the progeny trees of the spotted-fruit limb variations of the Thomson strain have been less productive thus far than the comparative progeny trees grown from buds secured from a normal branch of the parent tree.

The fruits borne by the two progeny trees propagated from the limb variation in the tree of the Thomson strain have all developed the characteristic brown spots that were shown by the fruits on the parent limb, except four fruits in the crop of 1923-24 on tree No. 1, which had normal Washington texture with ribbed surfaces. The progeny trees propagated from the normal part of the same parent tree have produced only normal Thomson fruits. Typical fruits from one of the progeny trees propagated from the spotted-fruit limb variation are shown in Plate 3, A, and typical fruits from one of the progeny trees propagated from a normal limb of the same parent tree are shown in Plate 3, B.

The progeny trees propagated from the limb variation of the tree of the Washington strain have produced a few normal unspotted fruits and a few brown-spotted yellow fruits with unspotted reddish orange sections in addition to the spotted fruits, as shown in Plate 4, A. These types are similar to those which have been occasionally found on the parent limb. Typical spotted fruits from the second progeny tree are shown in Plate 4, B. The number of fruits of these various types produced by the progeny trees is shown in Table III.

TABLE III.—*Types of fruit borne on the two progeny trees propagated from the spotted-fruit limb variation in a tree of the Washington strain*

Type of variations	Number of fruits produced									
	1920-21		1921-22		1922-23		1923-24		Totals	
	Tree No. 1	Tree No. 2	Tree No. 1	Tree No. 2	Tree No. 1	Tree No. 2	Tree No. 1	Tree No. 2	Tree No. 1	Tree No. 2
Brown-spotted.....	11	44	7	17	45	50	134	268	197	379
Brown-spotted with reddish-orange sections.....	0	0	4	17	1	6	20	20	25	43
Normal Washington.....	5	0	7	2	4	7	25	2	41	11
Washington texture and ribbed.....	1	0	2	0	2	0	11	1	16	1
Total fruits.....	17	44	20	36	52	63	190	291	279	434

The spotted oranges borne by the progeny trees ripen and drop prematurely and after the fruits drop the sunken brown spots increase in number and size, frequently covering a large portion of the surface of the fruits, in the same manner as is the case with the fruits borne by the parent limbs.

### CONCLUSION

The results of these progeny tests indicate that the brown-spotted and early-ripening characters of the fruits which have been described in the foregoing pages may occur as bud variations and that such limb variations may be perpetuated through bud propagation.

These studies suggest the probability that undesirable trees in established citrus orchards frequently may be due to unintentional propagations from similar limb variations.

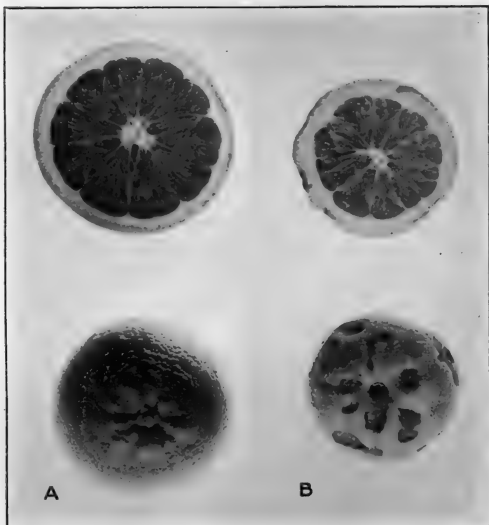
These progeny tests emphasize the fundamental importance of careful bud selection in the commercial propagation of citrus trees.

PLATE 1

A.—Typical brown-spotted, yellow oranges borne by a limb variation in the tree of the Thomson strain of the Washington Navel orange variety. Victoria ranch, Riverside, Calif. Photographed January 8, 1923. Negative 2160.

B.—Typical Thomson oranges borne by the normal limbs of the Thomson Navel orange tree. Victoria ranch, Riverside, Calif. Photographed January 8, 1923. Negative 2159.







## PLATE 2

Progeny orange trees propagated from (right) the limb variation which bears brown-spotted fruits and from (left) a normal branch of the same parent Thomson Navel tree. University of California Citrus Experiment Station, Riverside, Calif. Photographed January 5, 1924. Negative 2194.

### PLATE 3

A.—Typical brown-spotted yellow oranges borne by one of the progeny trees which was propagated from the limb variation which bears similar fruits in the tree of the Thomson strain of the Washington Navel orange. Both the trees of this progeny produce fruit like that shown above. University of California Citrus Experiment Station, Riverside, Calif. Photographed January 5, 1923. Negative 2132.

B.—Typical Thomson Navel oranges borne by one of the progeny trees which was propagated from a normal limb of the tree of the Thomson strain of the Washington Navel orange. The other tree in this progeny produces fruit of this same character. University of California Citrus Experiment Station, Riverside, Calif. Photographed January 5, 1923. Negative 2136.





#### PLATE 4

A.—Typical oranges produced by progeny tree No. 1, which was propagated from a limb variation which bears brown-spotted, yellow fruits in a tree of the Washington strain of the Washington Navel orange variety. Note the normal Washington fruit, the ribbed Washington fruit, fruits showing normal and ribbed Washington sections, and fruits showing the spotted condition in varying degrees. University of California Citrus Experiment Station, Riverside, Calif. Photographed January 5, 1924. Negative 2197.

B.—Typical brown-spotted yellow-colored oranges produced by progeny tree No. 2, which was propagated from the limb variation which bears brown-spotted fruits in a tree of the Washington strain of the Washington Navel orange variety. University of California Citrus Experiment Station, Riverside, Calif. Photographed December 17, 1923. Negative 2188.

# THE DETERMINATION OF NITRATE AND AMMONIA IN NITROGENOUS MATERIALS<sup>1</sup>

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## INTRODUCTION

The estimation of nitrate and of ammonia in the presence of other soluble and easily decomposable nitrogenous compounds of organic origin is difficult. They are determined by colorimetric methods or by procedures involving either gravimetric or volumetric features, and if present in large amounts the latter have proved more reliable. The present work consists mainly of a comparison of a recent Devarda alloy method devised by Strowd (6)<sup>3</sup> for the determination of nitrates in plants and a slight modification of same whereby the control determination in the modified method was used to estimate the ammoniacal nitrogen present in some samples. The latter has been made an incidental study, however, and the results obtained in this connection are included here, inasmuch as the procedure described may possibly be used for this determination on other materials.

## HISTORICAL REVIEW

It has been recognized from the earliest work on the subject that the estimation of nitrate in the presence of organic nitrogenous materials presents difficulties. Some colorimetric methods such as those devised by Caron (2), and particularly the modified phenolsulphonic acid procedure of Chamot and coworkers (3), have been found very satisfactory under certain conditions, provided the amount of nitrate present is small and a clear solution of it can be obtained. Other methods are more satisfactory when larger amounts of nitrate are present and among them some form of reduction process is generally used. Gasometric methods involving either the Schlösing method or modifications (7, p. 456) have found favor with some workers. The "nitron" method (7, p. 451) has also been found to be applicable in certain cases. If no separation of the nitrate is to be made, but it is desired to include it with other forms of nitrogen that may be present, one of the improved salicyl-sulphonic acid procedures may be used (1, p. 7, 8).

The methods that are most popular at present, however, involve some reduction process whereby the nitrate is reduced to ammonia by nascent hydrogen generated by the action of an alkali or acid on one or more metals. The ammonia is afterwards distilled into an excess of standard acid and titrated. Briefly mentioned, they are the ferrous sulphate-zinc-soda method, which is carried on in an alkaline solution and recommended for nitrogen in commercial nitrate; the zinc-iron method, also carried on in an alkaline solution; and the Ulsch-Street, or reduced iron method, in which the nitrate is reduced in an acid solution by the action of sulphuric acid on reduced iron. The above three methods have been adopted as official in fertilizer work by the Association of Official Agricultural Chemists (1, p. 10). Some other methods which have been used are reduction with aluminum in an alkaline solution according to Pozzi-Escot (5) and

<sup>1</sup> Received for publication April 1, 1924. Published by permission of the director of the Kentucky Agricultural Experiment Station.

<sup>2</sup> The writer desires to thank Dr. A. M. Peter, head of the Department of Chemistry, for helpful criticisms during this investigation and in the preparation of the manuscript.

<sup>3</sup> Reference is made by number (italic) to literature cited, p. 538, 539.



reduction with Devarda's alloy (50 parts copper, 45 parts aluminum, and 5 parts zinc), conducted in an alkaline solution and now known as the Devarda alloy method (7, p. 456). The last procedure with some modifications has been found to be very satisfactory by many workers, and it is now becoming more generally used. Further work, together with minor improvements, will probably extend its application.

### EXPERIMENTAL WORK

The writer, having occasion to make a large number of nitrate determinations in cured tobacco, desired to find a satisfactory method for this purpose. Previous experience had shown that if a distillation is made of tobacco with water and magnesium oxid according to the official procedure for ammoniacal nitrogen in fertilizers (1, p. 10), ammonia is slowly evolved from the nitrogenous compounds present and comes over long after a sufficient volume of distillate has been collected to account for any ammonia compound that may have been present originally.

The writer was most impressed with Strowd's method for the determination of nitrites and nitrates in plants (6) which is a modification of the Devarda alloy method. Strowd found that his procedure was more satisfactory for this purpose than any other method tried. One good point in this procedure is that it includes a control determination with each sample, without the Devarda alloy.

Strowd's procedure was first tried on some aqueous extracts of cured tobacco leaf and found that with some samples it was not always possible to get satisfactory duplicates. It was thought possible that the prescribed conditions do not permit the hydrogen to completely reduce the nitrate during the preliminary heating and subsequent distillation. The modification described below was afterwards found to usually give higher and more concordant results than Strowd's method. The procedure used in this work and subsequently referred to as the modified method is as follows:

### THE MODIFIED STROWD METHOD

Dilute two equal filtered portions of a cold-water extract of the sample to 250-350 cc. in 1-liter Kjeldahl flasks. Add a small piece of paraffin, a few small glass beads or pieces of pumice stone and 2.5-3 gm. of sodium hydroxid in solution (1:2) to each. To one solution add 2-3 gm. of Devarda alloy powder and use the other as a control. Place a rubber stopper with a Bunsen valve in the neck of each flask and let stand 24 hours, after which attach to a condenser carrying an improved form of distilling bulb (McHargue's form (4) was used in this work). Heat with a low flame just under boiling for 2 hours and then distill over at least 150 cc. The distillate is collected in a measured portion of 0.1 N sulphuric acid, the end of the outlet tube having been so placed as to dip beneath the acid from the beginning. Care should be taken that the control and determination be carried on in the same manner; that is, distilled at the same rate and same volume of distillate collected. The excess of acid in the receiving flask is then titrated with standard alkali, using an aqueous solution of sodium alizarin sulphonate as indicator. The difference between the two titrations corresponds to nitric plus nitrous nitrogen.

In this work, any small amounts of nitrite which may have been present are disregarded. It is doubtful if any quantity was present that would have materially influenced the nitrate results and besides this would not affect the comparisons between the two procedures. If its separation is desired, this can be made by a preliminary treatment with aspartic acid according to Strowd's directions and then following the above procedure. If relatively large amounts of nitrate

are to be determined, it is preferable to use the larger amounts of water and reagents specified and collect more distillate.

The principal change from Strowd's method is that the reduction is allowed to proceed for several hours before the solution is heated and the time of preliminary heating is increased. Some arrangement like the Bunsen valve permits the distilling apparatus to be utilized in the meantime for other purposes. The other modifications which prescribe a larger amount of alloy, a longer period of preliminary heating and larger volume of distillate, provide better conditions when relatively large amounts of nitrate are to be determined. The writer is of the opinion that the preliminary reduction before heating is the most important change made in Strowd's procedure, however, as excellent results have been obtained on some samples of moderate nitrate content by otherwise conforming to his directions.

Experiments have been made with the modified procedure to test the following points: (1) A possible loss of ammonia in the preliminary reduction; (2) its efficiency compared with the regular Strowd method for the reduction of relatively large amounts of pure nitrate; (3) the recovery of added nitrate in the presence of tobacco; (4) a comparison of the two procedures for nitrate in tobacco; (5) a comparison of the modified Strowd and the Ulsch-Street methods for nitrate in fertilizers and (6) a comparison of the nitrogen obtained in the control determinations by the modified Strowd and Ulsch-Street methods with that found by the official magnesium oxid method for ammoniacal nitrogen in fertilizers.

Blank determinations were made and deducted in reporting the results in the tables on all of the above work.

#### LOSS OF AMMONIA IN THE PRELIMINARY REDUCTION

For this work either Baker's analyzed ammonium sulphate or Eimer and Amend's tested ammonium chlorid were used. They were assumed to be pure, were practically moisture-free but no analyses were made to verify their composition inasmuch as they were to be used for a comparative study of the two methods for ammonia recovery. As the results obtained on both salts were equally close to the theoretical, no distinction is made in their discussion. For the determination, separate weighings of 0.4 gm. of the former salt or 0.3 gm. of the latter were used. These amounts are theoretically equivalent to 0.0848 gm. and 0.0785 gm. of nitrogen, respectively, representing more nitrate than would ordinarily be present in a determination. Control determinations were made by the modified method in which either the ammonium salt or Devarda alloy were omitted. When the ammonia salt was omitted the results were negligible, with or without the alloy. The recovery of ammonia from ammonium salts in the absence of the alloy is shown in Table I, C and E.

Determinations were also made by the modified method, connecting the flasks with the condenser instead of the Bunsen valve during the 24-hour digestion at room temperature. These are indicated in Table I, D and E. At the writer's request, duplicate determinations were made in the same manner by H. D. Spears, of this station, and his results were within the range given and are included in the averages under these headings in Table I.

The results by the modified method indicate a slightly better recovery of the ammonia by the use of the Bunsen valve compared with those where the flask was connected at once with the condenser as shown in B and D. Neither are as good, however, as those found by the Strowd method. In the determinations where the alloy was not used as shown in C and E, the results were just the reverse, in fact the average of those in E or with the condenser are better than those found by the Strowd method.

A titration of the acid through which the hydrogen gas from the digestion of the ammonium salt with sodium hydroxid and Devarda alloy had passed for 24 hours, before heat was applied, where the flask was connected with the condenser, showed a very small quantity of ammonia by a Nessler test. The amount obtained required less than 0.05 cc. of 0.1 N acid in the titration.

TABLE I.—Percentage recovery of nitrogen from ammonium salts

Method	No. of determinations	Maximum	Minimum	Average
A—Strowd method.....	4	98.4	97.8	98.1
B—Modified Strowd method, Bunsen valve.....	4	97.1	96.2	96.6
C—Same as B, but without Devarda alloy.....	3	97.9	97.1	97.4
D—Same as B, but connected with condenser <sup>a</sup> .....	12	97.6	92.9	95.7
E—Same as D, but without Devarda alloy.....	8	99.5	97.5	98.7

<sup>a</sup> Block tin condensers were used for all distillations. A battery of same was kindly furnished for this work by the Feed Control Laboratory of this station.

It would appear from Table I that for some unaccountable reason, the Devarda alloy had a detrimental effect on the ammonia recovery, as lower results were obtained in its presence.

THE EFFICIENCY OF THE TWO PROCEDURES FOR THE REDUCTION OF RELATIVELY LARGE AMOUNTS OF PURE NITRATE

Pure potassium nitrate and sodium nitrate were used and separate weighings of 0.25 gm. or 0.5 gm. were made, generally the latter amount. No material differences were found in the recovery of the nitrogen from either quantity and all are included in Table II. A comparison was again made by the modified method, connecting the flask with the condenser instead of the Bunsen valve during the 24-hour digestion at room temperature. The results are given in B and C of Table II.

TABLE II.—Percentage recovery of nitrogen of pure nitrate

Method	No. of determinations	Maximum	Minimum	Average
A—Strowd method.....	5	99.2	98.7	<sup>a</sup> 98.9
B—Modified Strowd method, Bunsen valve.....	8	99.4	97.4	98.4
C—Same as B, but connected with condenser.....	6	99.0	92.8	<sup>b</sup> 95.4

<sup>a</sup> One determination equivalent to 89.2 per cent recovery is not included.

<sup>b</sup> One determination equivalent to 90.9 per cent recovery is not included.

It will be observed that good results were obtained by both procedures on relatively large amounts of pure nitrate. The determinations by the modified method where the flasks were connected at once with the condenser are not as good as those with the Bunsen valve. This is contrary to what would be expected if there had been a loss of ammonia in the latter case.

## THE RECOVERY OF ADDED NITRATE IN THE PRESENCE OF TOBACCO

The plan of this experiment was to digest the cured, pulverized tobacco in distilled water for 30 minutes by shaking every minute during the first and last five-minute periods. It was then filtered and two aliquots, each representing 0.7 gm. of tobacco were withdrawn. To one was added either 0.25 gm. or 0.5 gm. of sodium or potassium nitrate. Both determinations were made simultaneously and the difference in the nitric nitrogen obtained represents that recovered from the added nitrate. No material differences were found in the different quantities and kinds of nitrate used. Some determinations, also, were made by connecting the flasks at once with the condenser and these are so designated in the table. The tobacco samples were selected for their low nitrate content. Three contained 0.04 per cent and No. 80078 only 0.05 per cent nitric nitrogen. The results obtained are given in Table III.

TABLE III—*Recovery of added nitrate in the presence of tobacco*

Number	Strowd method	Modified Strowd method	
		With Bun-sen valve	With con-denser
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
80078.....	97.1	97.4	91.3
	95.2	98.3	88.1
		95.1	
		97.3	
		97.5	
		96.5	
Average.....	96.2	97.0	89.7
80236.....	98.5	94.2	89.2
		97.7	
Average.....		96.0	
80247.....	99.3	98.3	88.3
		97.2	
Average.....		97.8	
80274.....	98.4	97.5	97.5
		95.5	
Average.....		96.5	

## A COMPARISON OF THE TWO PROCEDURES FOR NITRATE IN TOBACCO

Two aliquots prepared in the same manner as previously described, each representing 0.7 gm. of the cured sample, were withdrawn from the same filtered aqueous extract of the powdered tobacco. One aliquot was used for the determination and the other as a control, the latter having no Devarda alloy added. Some determinations, also, were made by the Strowd method in which the preliminary heating was for two hours instead of the prescribed one-hour period. The samples represent different grades of leaf, both of Burley and dark tobacco grown in Kentucky. The results obtained are given in Table IV.

TABLE IV.—Nitrate nitrogen in moisture-free tobacco by the two procedures

Number	Strowd method	Modified Strowd method
61339-----	<i>Per cent</i> 0. 17	<i>Per cent</i> 0. 18
	. 19	-----
	. 11	-----
	. 10	-----
Average-----	. 14	-----
61340-----	<sup>a</sup> . 11	. 15
61341-----	<sup>a</sup> . 11	. 15
61342-----	<sup>a</sup> . 02	. 07
61343-----	<sup>a</sup> . 17	. 29
61344-----	<sup>a</sup> . 04	. 06
61345-----	<sup>a</sup> . 05	. 16
61347-----	<sup>a</sup> . 22	. 24
61348-----	<sup>a</sup> . 03	. 06
61349-----	<sup>a</sup> . 16	. 19
61350-----	<sup>a</sup> . 01	. 08
61351-----	<sup>a</sup> . 03	. 06
61352-----	<sup>a</sup> . 11	. 22
61375-----	. 09	. 26
	<sup>a</sup> . 22	. 31
		. 31
		. 26
Average-----	. 16	. 29
61376-----	. 59	. 85
	. 77	. 80
	<sup>a</sup> . 78	. 89
		. 84
Average-----	. 71	. 85
61377-----	<sup>a</sup> . 15	. 20
61378-----	. 24	. 41
	<sup>a</sup> . 24	-----
Average-----	. 24	-----
61379-----	<sup>a</sup> . 12	. 20
61380-----	. 33	. 44
	<sup>a</sup> . 31	. 34
		. 44
		. 37
Average-----	. 32	. 40
80119-----	. 95	. 99
	. 88	. 99
Average-----	. 92	. 99
80125-----	1. 54	1. 36
	1. 42	1. 38
Average-----	1. 47	1. 37

<sup>a</sup> Preliminary heating was for 2 hours instead of 1 hour, as recommended.

TABLE IV.—Nitrate nitrogen in moisture-free tobacco by the two procedures—Con.

Number	Strowd method	Modified Strowd method
80132.....	<i>Per cent</i> 1. 23 1. 11	<i>Per cent</i> 1. 10 1 14
Average.....	1. 17	1. 12
80250.....	. 54 . 61	. 64 . 63
Average.....	. 58	. 64
80273.....	. 49 . 49	. 54 . 55
Average.....	. 49	. 55
80275.....	. 65 . 66	. 66 . 70
Average.....	. 66	. 68
80276.....	. 87 . 86	. 89 . 85
Average.....	. 87	. 87
80298.....	. 74 . 76	. 81 . 83
Average.....	. 75	. 82
80332.....	1. 04 1. 06	1. 06 1. 04
Average.....	1. 05	1. 05

Of the 28 samples used the modified method gave higher averages on 24, the same on 2, and lower on 2 than the other procedure.

#### COMPARISON OF THE MODIFIED STROWD AND ULSCH-STREET METHODS FOR NITRATE IN FERTILIZERS

The samples were obtained from the fertilizer department and were selected chiefly on account of their containing ammonium salts but no nitrate and part of their nitrogen in organic combination. All were complete fertilizers except Nos. 63902, 64026, and 64773, and these were, respectively, commercial nitrate of soda, commercial nitrate of lime, and pure bone meal. The total nitrogen content of the complete fertilizers varied from 0.91 per cent to 5.89 per cent and their organic nitrogen content from 0.47 per cent to 3.3 per cent. The object of selecting such samples was to find if the nitrogen of added nitrate could be recovered in their presence; if so, then this would afford a method for determining nitrate in the presence of ammonium salts and organic nitrogenous compounds. Moreover, it was thought that possibly the control determinations might also afford an accurate estimation of the ammoniacal nitrogen present.

This will be discussed later. Potassium nitrate was used and 0.25 gm. in solution was added in each determination, including the control.

When the filtered water extract of the sample was used the nitrate was added to this solution. Determinations were made on the whole sample and on the filtered aqueous extract of the same. In the latter aliquots were withdrawn from the same solution, one of which was used for the control. These are designated in the table as "Filtered." Those by the Ulsch-Street method were made in a similar manner and are likewise designated in the table. In all cases 0.7 gm. of fertilizer or aliquots representing this amount were used. The initial volume for the distillation by the modified Strowd method was 350 cc. and 3 gm. of Devarda alloy, together with the Bunsen valve, was used. The results are given in Table V.

TABLE V.—Comparison of the modified Strowd and Ulsch-Street methods for nitrate in fertilizers

Number	Modified Strowd method for recovery of added nitric N		Ulsch-Street method for recovery of added nitric N	
	Filtered	Not filtered	Filtered	Not filtered
63595.....	Per cent 98.6 97.1	Per cent 95.2 99.7	Per cent ----- -----	Per cent 93.9 -----
Average.....	97.9	97.5	-----	-----
63601.....	96.0 98.8	97.7 -----	<sup>a</sup> 95.1 -----	<sup>a</sup> 94.2 74.0
Average.....	97.4	-----	-----	84.1
63605.....	98.3 97.7	95.2 96.5	----- -----	92.5 -----
Average.....	98.0	95.9	-----	-----
63607.....	99.7 97.4 98.8	95.7 98.6 -----	----- ----- -----	86.7 ----- -----
Average.....	98.6	97.2	-----	-----
63619.....	98.6 98.6	96.2 99.7	----- -----	75.7 -----
Average.....	98.6	98.0	-----	-----
63621.....	98.0	99.1 97.1	----- -----	77.2 -----
Average.....	-----	98.1	-----	-----
63899.....	98.0 98.3	101.7 -----	<sup>a</sup> 102.9 -----	<sup>a</sup> 95.7 84.5
Average.....	98.2	-----	-----	90.1

<sup>a</sup> Volume of solution was 350 cc. and collected 150 cc. of distillate. In the official method the prescribed volume is less than 150 cc. and only 100 cc. of distillate could be obtained. The solution of acid and iron was boiled for 10 minutes instead of 5 minutes; as recommended.

TABLE V.—Comparison of the modified Strowd and Ulsch-Street methods for nitrate in fertilizers—Continued

Number	Modified Strowd method for recovery of added nitric N		Ulsch-Street method for recovery of added nitric N	
	Filtered	Not filtered	Filtered	Not filtered
63902.....	<i>Per cent</i> <i>b</i> 15. 87 <i>b</i> 15. 97 <i>d</i> 15. 92	<i>Per cent</i> <i>c</i> 16. 24 <i>c</i> 16. 10	<i>Per cent</i> ----- ----- -----	<i>Per cent</i> <i>c</i> 16. 10 <i>c</i> 15. 99
Average.....	-----	16. 17	-----	16. 05
64026.....	<i>b</i> 12. 80 <i>b</i> 12. 42 <i>d</i> 12. 28	<i>c</i> 12. 28 <i>c</i> 12. 23	----- ----- -----	<i>c</i> 11. 92 <i>c</i> 12. 19
Average.....	-----	12. 26	-----	12. 06
64773.....	100. 0 99. 1	98. 8 97. 7	<i>a</i> 90. 2 -----	<i>a</i> 95. 1 77. 5
Average.....	99. 6	98. 3	-----	86. 3
64776.....	96. 8 98. 9	97. 7 99. 1	----- -----	86. 1
Average.....	97. 9	98. 4	-----	-----
64777.....	98. 3 99. 7	98. 8 94. 2	----- -----	78. 0 79. 2
Average.....	99. 0	96. 5	-----	78. 6
64813.....	99. 1 96. 5 98. 6	97. 7 97. 7 98. 8	----- ----- -----	85. 3
Average.....	98. 1	98. 1	-----	-----
64828.....	98. 6 95. 4	97. 1 98. 3	----- -----	82. 4
Average.....	97. 0	97. 7	-----	-----

*b* Total nitrogen in original sample by Kjeldahl-Gunning-Arnold method was modified to include nitrate and CuSO<sub>4</sub> used but no Hg.

*c* Nitric nitrogen found in original sample by method indicated.

*d* Nitric nitrogen found in original sample by the fertilizer department according to the ferrous sulphate-zinc-soda method.

There was a slightly better recovery of the nitrate by the Strowd method but the results by both procedures are good except in those determinations where the flasks were connected at once with the condenser, in which case the results were lower. Here, as in the experiments where the nitrate alone was used, the results were contrary to what would be expected if there had been a loss of ammonia through the Bunsen valve.

The results show that a better recovery of the nitrate was made by the modified Strowd than by the Ulsch-Street method. A slight modification of the latter method to provide a better reduction and distillation proved of decided advantage. This is shown in those determinations where the reduction was for 10



minutes and a larger volume of distillate was collected. There were not very large differences found by the modified Strowd method in the percentages of nitrate recovered from the filtered and unfiltered solutions of tobacco. The advantage is slightly in favor of the filtered solution. Good results were obtained in either case, and this would indicate that it is not necessary to filter in order to make a satisfactory determination.

The modified Strowd and Ulsch-Street methods were found to be very satisfactory for commercial nitrates, but the latter method gave much lower results on the other samples except when the modification of it referred to above was employed, in which event more satisfactory results were obtained.

COMPARISON OF THE MODIFIED STROWD, ULSCH-STREET, AND OFFICIAL MAGNESIUM OXID METHODS FOR AMMONIACAL NITROGEN IN FERTILIZERS.

In this work the results obtained in the control determinations by the modified Strowd method on the fertilizer samples were compared with those found for ammoniacal nitrogen by the official magnesium oxid method. A more limited comparison was also made in the same manner by the Ulsch-Street method. (See Table VI.)

TABLE VI.—Comparison of the modified Strowd, Ulsch-Street and official magnesium oxid methods for ammoniacal nitrogen in fertilizers

Number	Official MgO method	Modified Strowd method		Ulsch-Street method	
		Filtered	Not filtered	Filtered	Not filtered
63595	1. 12 b 1. 14	Per cent	Per cent	Per cent	Per cent
		1. 06	1. 25	-----	1. 33
		1. 11	1. 26	-----	-----
		1. 11	1. 26	-----	-----
		1. 02	-----	-----	-----
Average	1. 13	1. 08	1. 26	-----	-----
63601	0. 28 b 0. 21	. 29	. 50	c 0. 32	c . 44
		. 31	. 53	-----	. 45
		. 23	. 43	-----	. 48
		-----	-----	-----	-----
Average	0. 25	. 28	. 49	-----	. 46
63605	0. 42 b 0. 37	. 36	. 68	-----	. 75
		. 35	. 72	-----	-----
		. 35	. 64	-----	-----
		. 30	-----	-----	-----
		-----	-----	-----	-----
Average	0. 40	. 34	. 68	-----	-----
63607	1. 17 b 1. 24	1. 21	1. 35	-----	1. 37
		1. 20	1. 33	-----	-----
		1. 25	1. 29	-----	-----
		1. 17	-----	-----	-----
		-----	-----	-----	-----
Average	1. 21	1. 21	1. 32	-----	-----

a Control computed as ammoniacal nitrogen.  
b Results obtained by this method in the fertilizer department.  
c Volume of solution was 350 cc. and collected 150 cc. of distillate. In the official method the prescribed volume is less than 150 cc. and only 100 cc. of distillate could be obtained. The solution of acid and iron was boiled for 10 minutes instead of 5 minutes as recommended.

TABLE VI.—Comparison of the modified Strowd, Ulsch-Street and official magnesium oxid methods for ammoniacal nitrogen in fertilizers—Continued

Number	Official MgO method	Modified Strowd method		Ulsch-Street method	
		Filtered	Not filtered	Filtered	Not filtered
63619.....	0. 44 <sup>b</sup> 0. 44	<i>Per cent</i> 0. 41 . 37 . 41 . 40	<i>Per cent</i> 0. 65 . 62 . 60	<i>Per cent</i> ----- ----- ----- -----	0. 64 ----- ----- ----- -----
Average.....	0. 44	. 40	. 62	-----	-----
63621.....	0. 78 <sup>b</sup> 0. 76	. 81 . 85 . 81 . 81	. 89 . 89 . 84	-----	. 87 ----- ----- ----- -----
Average.....	0. 77	. 82	. 87	-----	-----
63899.....	4. 54 <sup>b</sup> 4. 48	4. 64 4. 59 4. 60	4. 60 4. 72 4. 72	<sup>c</sup> 3. 96 ----- -----	<sup>c</sup> 4. 54 4. 56 ----- ----- -----
Average.....	4. 51	4. 61	4. 68	-----	4. 55
64773.....	0. 18	. 23 . 26 . 25 . 13	. 38 . 40 . 41 . 27	<sup>c</sup> . 21 ----- ----- -----	<sup>c</sup> . 30 . 33 . 27 ----- -----
Average.....	-----	. 22	. 37	-----	. 30
64776.....	0. 45 <sup>b</sup> 0. 45	. 47 . 46 . 44	. 63 . 61 . 55	----- ----- -----	. 51 . 53 ----- ----- -----
Average.....	0. 45	. 46	. 60	-----	. 52
64777.....	0. 79 <sup>b</sup> 0. 77	. 85 . 83 . 83 . 82	1. 00 . 97 1. 01 . 92	----- ----- ----- -----	. 94 . 85 ----- ----- -----
Average.....	0. 78	. 83	. 98	-----	. 90
64813.....	0. 96 <sup>b</sup> 0. 93	. 95 . 93 . 92 . 91 . 91	1. 04 1. 04 1. 07 1. 03	----- ----- ----- -----	. 88 1. 04 ----- ----- -----
Average.....	0. 95	. 92	1. 05	-----	. 96
64828.....	0. 15	. 16 . 17 . 15 . 13	. 35 . 29 . 28	----- ----- ----- -----	. 29 ----- ----- ----- -----
Average.....	-----	. 15	. 31	-----	-----

<sup>b</sup> Results obtained by this method in the fertilizer department.<sup>c</sup> Volume of solution was 350 cc. and collected 150 cc. of distillate. In the official method the prescribed volume is less than 150 cc. and only 100 cc. of distillate could be obtained. The solution of acid and iron was boiled for 10 minutes instead of 5 minutes as recommended.

The results obtained for ammoniacal nitrogen by the modified Strowd method show that it is necessary to filter the solution, after which the aliquot for the control can be used for the ammonia determination. In fact the duplicates agree as well as the determinations made by different analysts by the official method on the same sample.

The distillation with magnesium oxid for ammoniacal nitrogen often gives high results on materials containing organic nitrogen. As the averages obtained by the modified Strowd method for this determination agree with the official procedure consequently they together with the nitrate results indicate that it is possible to make a satisfactory estimation of both ammonia and nitrate on the same solution provided a filtered aqueous extract of the sample is employed. If only the nitrate is desired, filtration will probably not be necessary although it may be desirable under different conditions.

The writer does not recommend the modified Strowd procedure for all nitrate determinations until further work establishes its reliability for the purpose. At the same time, it is believed that it will be found useful for samples of a different nature from those employed in this investigation.

### SUMMARY

1. A comparison has been made of a Devarda alloy method published by Strowd for the determination of nitrates in plants and a modification of same in which it was found advantageous to let the reduction continue for twenty-four hours at room temperature, previous to heating the solution.

2. Both procedures show a fairly complete recovery of considerable amounts of pure nitrate in solution and of nitrate added to tobacco of a low nitrate content.

3. More concordant and generally higher results were obtained, however, by the modified Strowd method when nitrates were determined in different kinds of tobacco of variable nitrate content.

4. The modified method was also found to be applicable for the estimation of nitrate in commercial fertilizers. No determinations were made by the regular Strowd procedure on these samples.

5. The control determination which is necessary as a check in the modified procedure was found to be as accurate for the determination of ammoniacal nitrogen in fertilizers as the official magnesium oxid method.

6. If both ammonia and nitrate are to be determined from aliquots of the same aqueous extract of a sample, it is necessary to filter from the residue. Where nitrate alone was determined in the samples used in this work, previous filtration proved to be of small advantage.

7. The modified Strowd method is not recommended for general application for nitrate determinations until its reliability is established, but it is believed that its use can be extended to others of a different nature from those employed in this investigation.

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## PUCCINIA GRAMINIS POAE ERIKSS. AND HENN. IN THE UNITED STATES<sup>1 2</sup>

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Eriksson and Henning (4),<sup>3</sup> in 1896, first described *Puccinia graminis poae* on *Poa chaixii* Vill. Eriksson (2) later showed that it could infect *Poa caesia* Smith, *P. compressa* L. and *P. nemoralis* L., and that aecia were formed on *Berberis vulgaris* L. The following grasses were inoculated by Eriksson but did not become infected: *Briza media* L., *Festuca elatior* L., *F. ovina* L., *F. pilosa* Haller, *Hordeum vulgare* L., *Koeleria cristata* var. *gracilis* A. Gray and *Phleum boehmeri* Wibel. Jaczewski (5) recorded the occurrence of this form on *Poa compressa* L. in Russia, and stated that it also could infect *P. pratensis* L. and *P. serotina* Ehrh., but not *Agropyron repens* (L.) Beauv., *Avena sativa* L., *Bromus inermis* Leyss., *B. secalinus* L., *Dactylis glomerata* L., *Hordeum vulgare* L., *Secale cereale* L., and *Triticum vulgare* Vill. Both Eriksson and Jaczewski called *P. graminis poae* "scharf fixiert" and Eriksson designated it as "Isophage," because it was confined to a single genus, *Poa*.

The writers have not been able to find records of the occurrence of *P. graminis poae* in the United States. Neither Carleton (1) nor Stakman and Piemeisel (14) mentioned it; and the writers did not find it during the course of extensive field observations previous to 1923. Several specimens of what appeared to be *P. graminis* on *Poa* spp. have been sent to the writers, but the amount of rust always was small and no infection was obtained as a result of artificial inoculations until inoculations were made with material obtained from Michigan in the fall of 1922.

In the fall of 1922 Donald G. Fletcher and Walter F. Reddy observed very heavily infected *Poa compressa* near barberry bushes in the vicinity of Pontiac, Mich., and sent material to the writers for identification. There were few urediniospores on the plants, the rust being mostly in the telial stage.

In order definitely to establish the identity of the rust inoculation experiments were made. On account of the paucity of uredinial material it was necessary to start with teliospores. The results of these experiments are given in Table I. In the summer of 1923 plants heavily infected with the uredinial stage were collected and additional inoculations made, the results of which are given in Table II.

<sup>1</sup> Received for publication March 12, 1924.

<sup>2</sup> Cooperative investigations between the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, and the Department of Agriculture of the University of Minnesota. Published with the approval of the Director, as Paper No. 426 of the Journal Series of the Minnesota Agricultural Experiment Station.

<sup>3</sup> Reference is made by number (italic) to "Literature cited," pp. 547-548

TABLE I.—Results of inoculating *Berberis vulgaris* and various grasses and cereals at University Farm, St. Paul, Minn., with *Puccinia graminis poae*, from *Poa compressa* collected near Pontiac, Mich., in 1922 <sup>a</sup>

Original inoculum	Species and varieties inoculated—			
	March 21, 1923	April 26, 1923	May 18, 1923	June 5, 1923
Teliospores from <i>Poa compressa</i> .	<i>Berberis vulgaris</i> ... 4—	<i>Poa annua</i> ... 0 <i>P. compressa</i> 4— <i>P. nemoralis</i> 0 <i>P. pratensis</i> 4 <i>P. triflora</i> ... 0	<i>Poa annua</i> ..... 0 <i>P. compressa</i> ..... 4— <i>P. nemoralis</i> ..... 4 <i>P. pratensis</i> ..... 4 <i>P. triflora</i> ..... 0 <i>Avena sativa</i> (Victory, Minn. 514) <sup>b</sup> ..... 0 <i>Hordeum vulgare</i> (Manchuria, Minn. 105)..... 0 <i>Secale cereale</i> (Rosen, Minn. 82)..... 0 <i>Triticum compactum</i> (Little Club, C.I. 4066) <sup>a</sup> 0	<i>Poa compressa</i> ... 4 <i>Agrostis alba</i> ..... 0 <i>Dactylis glomerata</i> ..... 0 <i>Secale cereale</i> (Rosen, Minn., 82)..... 0

<sup>a</sup> The sequence of inoculations in Tables I and II is indicated by dashes, proceeding from left to right. All of the hosts in the same vertical column were inoculated with spores from the host immediately preceding the dashes. The degree of infection is indicated after each plant inoculated: 0=no infection; 1=extremely light infection; 3=moderate infection; 4=very heavy infection. The date above each vertical column is that on which the inoculations were made.

<sup>b</sup> Accession numbers of Minnesota Agricultural Experiment Station, and Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, respectively.

TABLE II.—Results of inoculating *Poa* spp., *Phleum pratense*, and cereals with urediniospores of *Puccinia graminis poae* from *Poa compressa* collected near Pontiac, Mich., in 1923

Original inoculum	Species and varieties inoculated—			
	August 23, 1923	September 6, 1923	September 28, 1923	October 18, 1923
Urediniospores from <i>Poa compressa</i> .	<i>Poa annua</i> ..... 3 <i>P. compressa</i> 3— <i>P. fertilis</i> ..... 3 <i>P. nemoralis</i> ... 3 <i>P. triflora</i> ..... 3 <i>Avena sativa</i> (Victory, Minn. 514)..... 0 <i>Phleum pratense</i> ..... 0 <i>Triticum compactum</i> (Little Club, C.I. 4066) <sup>a</sup> ..... 0	<i>Poa compressa</i> 4—        <i>P. pratensis</i> 4—	<i>Poa compressa</i> ..... 4— <i>Avena sativa</i> (Victory, Minn. 514) <sup>a</sup> ..... 0 <i>Hordeum vulgare</i> (Manchuria, Minn. 105)..... 1 <i>Phleum pratense</i> ..... 0 <i>Secale cereale</i> (Rosen, Minn. 82)..... 0 <i>Triticum compactum</i> (Little Club, C. I. 4066)..... 0  <i>Poa compressa</i> ..... 4 <i>Phleum pratense</i> ..... 0	<i>Poa compressa</i> 3 <i>P. pratensis</i> ..... 4

<sup>a</sup> Accession numbers of Minnesota Agricultural Experiment Station, and Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, respectively.

It will be seen from Tables I and II that *Poa annua* L., *P. compressa* L., *P. fertilis* Host, *P. nemoralis* L., *P. pratensis* L., and *P. triflora* Gilib. are susceptible to the rust. Very heavy infection always resulted from inoculating *P. compressa*. While the infection on the other species of *Poa* was normal, it was not so heavy as that on *P. compressa*. *Agrostis alba* L., *Dactylis glomerata* L., and *Phleum pratense* L. did not become infected, so the rust could not have been either *P. graminis agrostis* or *P. graminis phleipratensis*. In addition, the fact that *Berberis vulgaris* was very susceptible also excluded the possibility of the rust being *P. graminis phleipratensis*. As wheat, oats, and rye apparently are immune, and barley nearly so, the rust could not be the *tritici*, the *avenae* or the *scalis* variety. It therefore must be *P. graminis poae*.

The host range of *P. graminis poae* appears to be narrow. According to Eriksson (2 and 3) and Jaczewski (5), this rust variety is restricted to the genus *Poa*. If this is true it is the only variety of *P. graminis* now known to occur in the United States which infects species of a single genus only. However, it probably can infect, at least weakly, members of other genera, because minute uredinia developed on barley (Table II). Extensive inoculations are now under way in order to ascertain the infection capabilities of the rust.

Morphologically, also, the variety *poae* is quite distinct, the spores being characteristically small. The urediniospores, teliospores and aeciospores are considerably shorter than those of any other variety, but they do not differ so much in width. Tables III and IV present the measurements of the three kinds of spores of *P. graminis poae* and a comparison of these with those of the other varieties of *P. graminis*.

TABLE III.—Variations and constants for length and width of the various spores of *Puccinia graminis poae* grown on congenial host plants and under favorable environmental conditions

Kinds of spores	Spore diameters	Spore classes					n	Mean	Standard deviation	Coefficient of variability		
Teliospores from <i>Poa compressa</i> ---	Length.	20μ	30μ	40μ	50μ	60μ	100	36.90±0.62	9.13±0.44	24.74±1.25		
		8	35	40	14	3						
	Width.	11μ	13μ	15μ	17μ	19μ	21μ	100	15.52±0.14	2.07±0.10	13.34±0.65	
		4	19	35	33	7	2					
Aeciospores from <i>Berberis vulgaris</i> ..	Length.	12μ	13μ	14μ	15μ	16μ	17μ	18μ	100	15.07±0.09	1.35±0.06	8.96±0.43
		2	11	20	31	21	11	4				
	Width.	11μ	12μ	13μ	14μ	15μ			100	13.23±0.07	0.97±0.05	7.33±0.35
		5	14	43	29	9						
Urediniospores from <i>Poa compressa</i> -----	Length.	15μ	17μ	19μ	21μ	23μ	100	18.64±0.10	1.51±0.07	8.10±0.39		
		1	35	46	17	1						
	Width.	13μ	14μ	15μ	16μ	17μ	18μ	100	15.78±0.07	1.06±0.05	6.72±0.32	
		3	12	14	48	21	2					

TABLE IV.—Summary of differences between the means of the dimensions of the various spores of *Puccinia graminis poae* and those of other varieties of *Puccinia graminis*

Diameters.	Biologic forms compared. <sup>a</sup>	Teliospores		Aeciospores		Urediniospores	
		Difference in means (in microns)	Difference divided by P. E.	Difference in means (in microns)	Difference divided by P. E.	Difference in means (in microns)	Difference divided by P. E.
Length	<i>P. graminis tritici</i> .....	14.90±0.79	18.86	4.65±0.21	22.14	13.76±0.21	65.52
	<i>P. graminis secalis</i> .....	10.45±0.77	13.57	2.03±0.21	9.67	8.50±0.17	50.00
	<i>P. graminis avenae</i> .....	9.25±0.75	12.33	3.55±0.14	25.36	9.86±0.18	54.78
	<i>P. graminis phleipratensis</i> ..	4.40±0.70	6.29			5.31±0.16	33.19
	<i>P. graminis agrostis</i> .....	3.40±0.74	4.59	1.39±0.20	6.95	3.73±0.16	23.31
Width	<i>P. graminis tritici</i> .....	1.15±0.18	6.39	2.43±0.12	20.25	4.01±0.09	44.56
	<i>P. graminis secalis</i> .....	0.75±0.18	4.17	0.20±0.13	1.54	1.41±0.09	15.67
	<i>P. graminis avenae</i> .....	0.32±0.18	1.78	1.47±0.12	12.25	4.16±0.10	41.60
	<i>P. graminis phleipratensis</i> ..	0.11±0.17	0.65			1.10±0.09	12.22
	<i>P. graminis agrostis</i> .....	0.88±0.18	4.89	0.25±0.11	2.27	0.10±0.09	1.11

<sup>a</sup> Data on forms other than *P. graminis poae* from Levine (6).



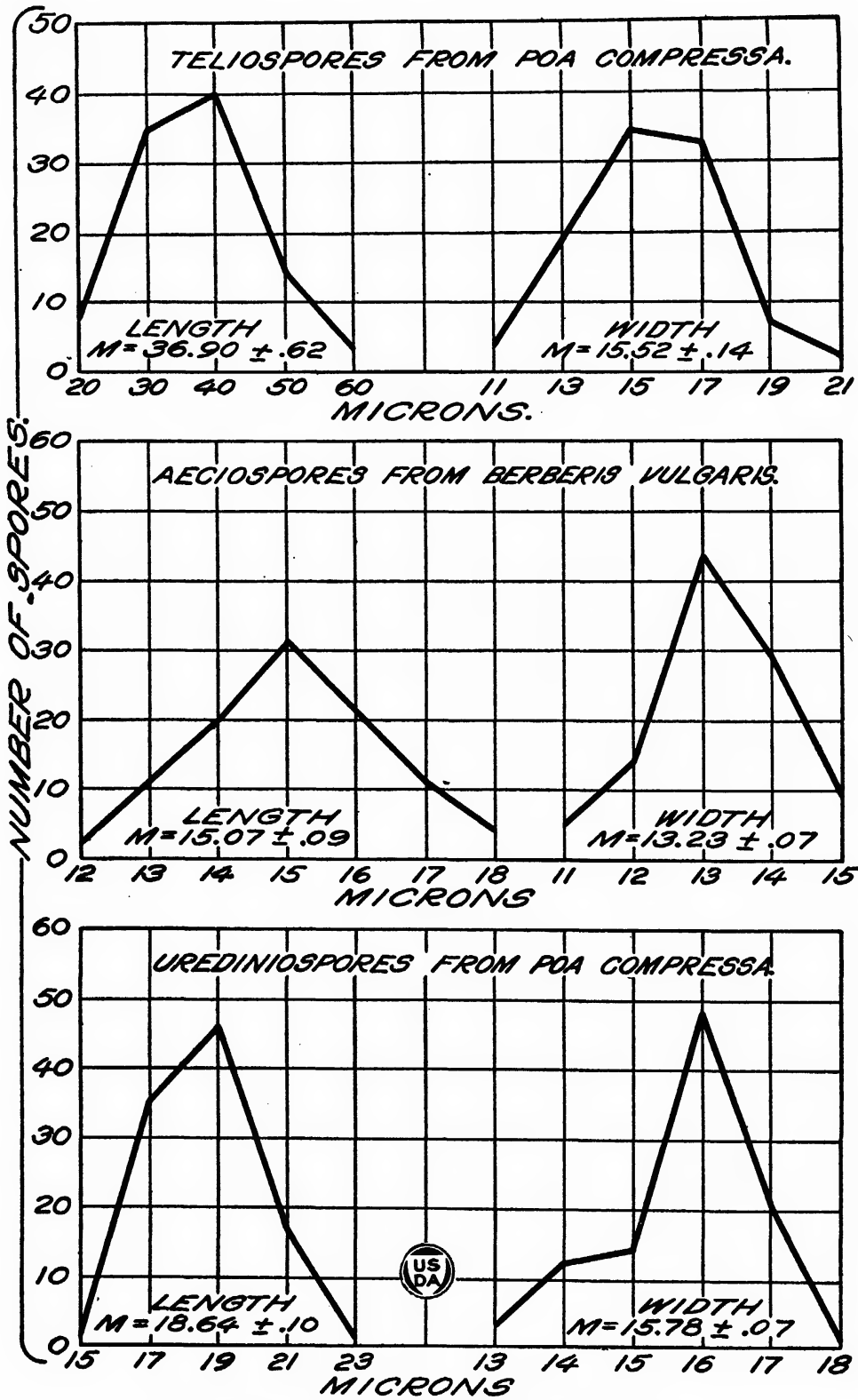


Fig. 1.—Variations in the size of spores of *Puccinia graminis poae*

The mean length for teliospores of *P. graminis poae* is  $3.40 \pm 0.74\mu$  less than that for those of *P. graminis agrostis*, which formerly were considered the shortest of all. This difference is 4.59 times the probable error and therefore is significant statistically. According to the computations of Pearl and Miner (8), the odds against the occurrence of such a difference on account of random sampling are about 520 to 1. The mean length for aeciospores of *P. graminis poae* is only  $1.39 \pm 0.20\mu$  less than that for those of the variety *agrostis*. But this difference is even more significant than the apparently greater difference between the means of the length of the teliospores of these varieties, because it is almost 7 times its probable error, and the odds against its chance occurrence are about 40,000 to 1. Of still greater significance is the difference between the means of the length of the urediniospores of these two varieties. It is more than 23 times its probable error, the actual difference being  $3.73 \pm 0.16\mu$ .

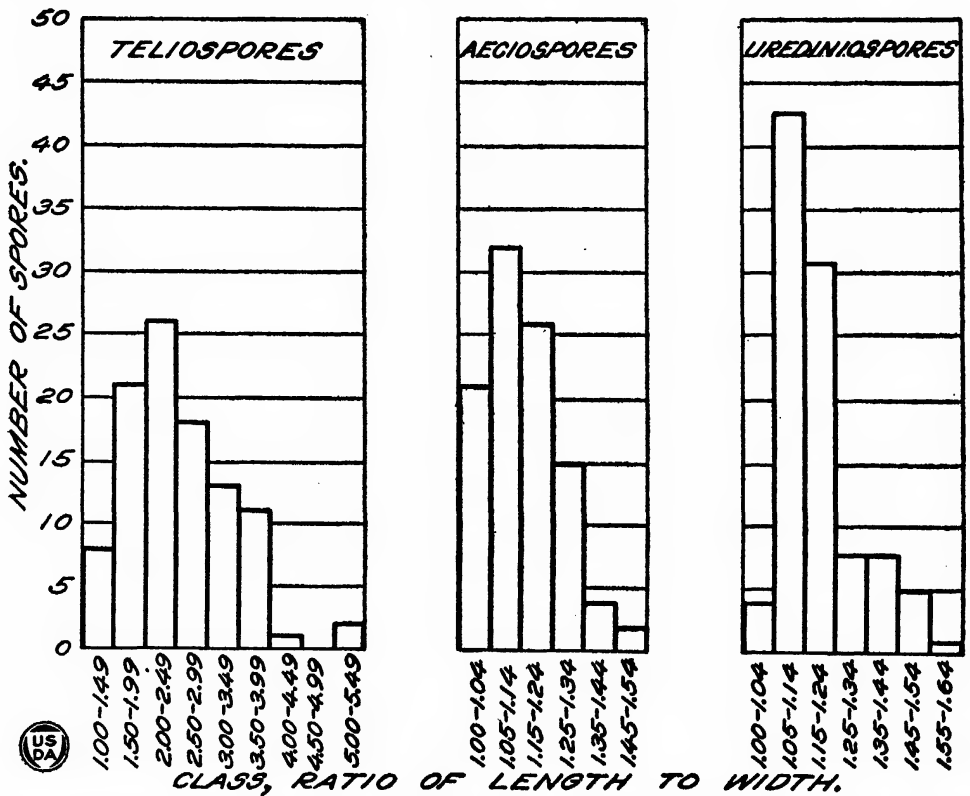


FIG. 2.—Ratios of length to width of the various spores of *Puccinia graminis poae* arranged in classes

The mean width of the teliospores of the *poae* variety is approximately the same as that of the varieties *avenae* and the *phleipratensis*, that of the aeciospores is similar to the mean width of the varieties *secalis* and *agrostis*, and that of the urediniospores is nearly the same as that of *P. graminis agrostis*.

The variations in length and width of the various spores of *Puccinia graminis poae* are shown graphically in fig. 1.

The ratio of length to width, as shown in fig. 2, was determined by the method suggested by Rosenbaum (10, p. 250). The correlations of length and width of the three different kinds of spores are presented in Tables V, VI, and VII.

The teliospores (Pl. 1, B) of the variety *poae* appear to be extremely variable in shape. Some are very short and broad ( $27.14 \times 20.01\mu$ ) while others are greatly elongated and narrow ( $63.48 \times 11.73\mu$ ). The predominating ratio of length to width of the teliospores is about 2 to 1. There is a definite negative correlation ( $-.613 \pm .0421$ ) between the long and short diameters of these spores (Table V).

The aeciospores (Pl. 1, D) are predominantly spherical to subspherical in shape, the modal length-to-width ratio being approximately 1.1 to 1. The thickening of the upper wall, in vertical section of the sorus, described by Rosen and Kirby (9) as characteristic of the aeciospores of *P. graminis*, was quite evident. There is some indication of a positive correlation ( $.194 \pm .0649$ ) between the length and width of these spores (Table VI), but it probably is too small to be considered statistically significant.

There is clearly no correlation ( $.038 \pm .0673$ ) between the two diameters of the urediniospores (Table VII). These spores are for the most part subglobose (Pl. 1, F), resembling the urediniospores of *P. graminis avenae* on *Bromus tectorum* L. (11, p. 62), and the predominating length-to-width ratio is about 1.1 to 1.

TABLE V.—Correlation table for 100 teliospores of *Puccinia graminis poae*. Spore length, subject; spore width, relative. Coefficient of correlation =  $-.613 \pm .0421$

		SPORE WIDTH						
		11μ	13μ	15μ	17μ	19μ	21μ	
SPORE LENGTH	20μ				5	2	1	8
	30μ	1		13	17	3	1	35
	40μ	1	9	17	11	2		40
	50μ		10	4				14
	60μ	2		1				3
		4	19	35	33	7	2	100

TABLE VI.—Correlation table for 100 aeciospores of *Puccinia graminis poae*. Spore length, subject; spore width, relative. Coefficient of correlation =  $.194 \pm .0649$

		SPORE WIDTH					
		11μ	12μ	13μ	14μ	15μ	
SPORE LENGTH	12μ	1	1				2
	13μ		2	9			11
	14μ		3	9	8		20
	15μ	2	4	11	11	3	31
	16μ	1	3	10	5	2	21
	17μ	1	1	3	4	2	11
	18μ			1	1	2	4
		5	14	43	29	9	100

TABLE VII.—Correlation table for 100 urediniospores of *Puccinia graminis poae*. Spore length, subject; spore width, relative. Coefficient of correlation = .038  $\pm$ .0673

		SPORE WIDTH					
		13 $\mu$	14 $\mu$	15 $\mu$	16 $\mu$	17 $\mu$	18 $\mu$
SPORE LENGTH	15 $\mu$		1				1
	17 $\mu$	2	2	9	18	4	35
	19 $\mu$	1	4	1	25	13	46
	21 $\mu$		4	4	5	4	17
	23 $\mu$		1				1
		3	12	14	48	21	2
		100					

The varieties of *P. graminis* now known in the United States are as follows:<sup>4</sup> *P. graminis tritici* Erikss. and Henn. (actually consists of many forms or races), *P. graminis secalis* Erikss. and Henn. (consists of several forms), *P. graminis avenae* Erikss. and Henn. (consists of several forms), *P. graminis phleipratensis* (Erikss. and Henn.) Stak. and Piem., *P. graminis agrostis* Erikss., and *P. graminis poae* Erikss. and Henn. The first three must be considered as group varieties, consisting of many forms which can be differentiated by their parasitic action on varieties of *Triticum* spp., *Secale cereale*, and *Avena* spp., respectively (12, 7, and 13).

In addition to these the following have been described by Eriksson (3, p. 370-371) and by Jaczewski (5, p. 353) in Europe: *P. graminis airae* Erikss. and Henn., *P. graminis calamagrostis* Jacz. (*P. graminis epigeii* Erikss.), *P. graminis aperae* Jacz. and *P. graminis arrhenatheri* Jacz. [*P. graminis avenae* Erikss. and Henn., according to Eriksson (3, p. 370)].

It is not known how widely *P. graminis poae* is distributed in the United States. It has been found abundantly only near barberry bushes. The epidemics on *Poa compressa* in 1922 and 1923 near Pontiac, Mich., clearly started from rusted barberries. Heavily rusted *P. compressa* also was found in 1923 near rusted barberry bushes in the vicinity of North Vernon, Ind.<sup>5</sup>

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## PLATE 1

*Puccinia graminis poae* on congenial host plants, and photomicrographs of the different spore forms (all photomicrographs x 500).

A.—Heavily rusted *Poa compressa* from the field; the rust is in the telial stage.

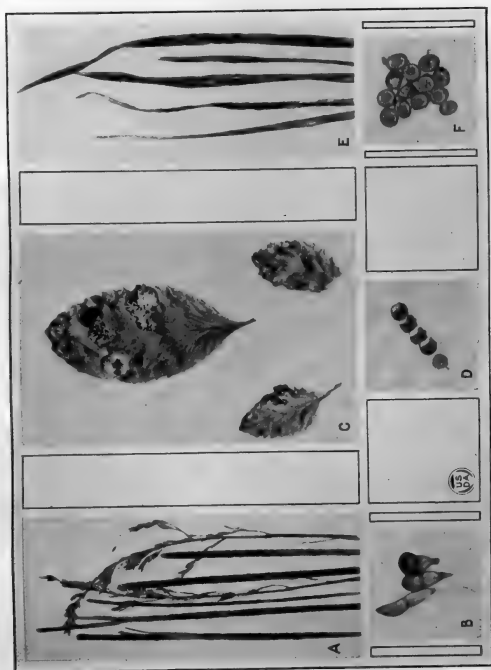
B.—Teliospores from A.

C.—Aecia on *Berberis vulgaris* resulting from artificial inoculation in the greenhouse; note the long aecial cups.

D.—Aeciospores from C.

E.—*Poa compressa* heavily infected with the uredinial stage as a result of artificial inoculation with urediniospores in the greenhouse.

F.—Urediniospores from E.



# RELATION OF SOIL TEMPERATURE AND SOIL MOISTURE TO INFECTION BY PLASMODIOPHORA BRASSICAE<sup>1</sup>

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## INTRODUCTION

The peculiar nature of the clubroot disease of crucifers has focused upon it the attention of both mycologists and plant pathologists ever since Woronin first proved (21)<sup>2</sup> that it is caused by the parasitic slime mold. As a result there is an extensive literature dealing with both the scientific and the practical aspects of this malady. The life history of the organism is now very well understood and the recent studies by Kunkel (12) seem to give a satisfactory explanation of its relation to the host tissue. These more fundamental scientific studies seem to have proceeded more satisfactorily than those dealing with control measures. Following the early lead of Halsted (7, 8) American phytopathologists have generally relied on the use of lime as an adequate control measure, and Ravn (14, 15) has been an earnest advocate of liming for this disease in Europe. The results, while justifying the use of lime, have not proven uniformly reliable in either America (9) or Europe. Moreover plants that show early infection will sometimes recover (6) and produce a satisfactory crop.

The evidence seems to indicate that while soil reaction is important in governing infection with this parasite there must be other factors exercising an important and, perhaps at times, a controlling influence upon either its entrance into the host tissue or upon the subsequent development of the disease. It seems evident, therefore, that a fuller and more exact understanding of the factors influencing infection and the progress of the disease may contribute both to our understanding of the physiological relation of parasite and host and to the furthering of reliable control measures.

In this disease we are dealing with an organism which persists for some time in the soil and the attacks of which are practically confined to the underground host organs. The recent studies at Wisconsin (11, 19) upon the influence of environment upon other root diseases suggest that soil temperature and soil moisture may be expected to play an important part in determining the occurrence or severity of such diseases. The present paper deals with the results of efforts to determine the influence of these two factors.

## EARLIER OBSERVATIONS

Differences in the prevalence and severity of clubroot have frequently been attributed to variations in "climatic conditions," but few attempts have been made to analyze these conditions as to their effect upon the disease. Clubroot is reported as causing most damage in northern regions of the United States and Europe, from which it might be inferred that temperature plays an important part in its distribution. It has long been recognized that affected plants are more likely to wilt and die in periods of hot, dry weather during July and August, than at other times. This has been interpreted as the result of increased trans-

<sup>1</sup> Received for publication February 1, 1924.

<sup>2</sup> Reference is made by number (*italic*) to literature cited, p. 559-561.



piration at a time when the roots could not supply sufficient moisture because of the distortions due to the presence of the organism in their tissues. In interpreting this evidence it should be kept in mind that the parasite probably made its entry early in the host's development when the soil was relatively cool and moist, and that the disease had progressed to an advanced stage before the dry, hot period of midsummer. Halstead (7, 8) after noting the greater destructiveness of clubroot on turnips during a hot season comments as follows:

The extreme heat of midsummer, although detrimental to the normal development of turnips, seemed to have an opposite effect upon the parasite preying upon their roots. When harvested, the turnips were found to be distorted to a much greater degree than any grown upon the same land since the experiment began.

These observations, although directing attention to temperature, were based upon field plots where the comparisons were between different seasons in which other factors may have varied greatly.

Chupp (5) studied the earlier stages of the disease under controlled conditions and from his observations concluded that temperature might play an important part in spore germination and infection. In discussing his results on spore germination he states that—

Temperature conditions also influence germination of the spores. It was practically impossible to obtain infection in the greenhouse during the coldest winter months when the temperature was from 10° to 18° C. The spores also fail to germinate at ordinary room temperatures (from 16° to 21° C.) The optimum temperature for germination proved to be from 27° to 30° C. This, however, is not the case when spores are placed in test tubes on agar with young cabbage seedlings, for under such conditions infection takes place at a temperature of from 16° to 21° C. The presence of the host seems in some manner to exert an influence which to a certain extent takes the place of that offered by a greater amount of heat.

Chupp's results indicate that studies upon the germination of spores apart from the plant will not necessarily apply to conditions as they exist in the soil. The introduction of cabbage seedlings indicates that his first results were influenced more by some unfavorable condition of his germinating medium than by temperature. This seems to be the only case in which temperature has been definitely designated as a limiting factor in the development of clubroot.

The influence of soil moisture upon clubroot development has been given more attention by previous workers than has temperature. However, in these cases no one has clearly differentiated the possible influence of moisture from the other variable factors such as soil reaction, aeration, or humus content. Chupp (5), Reed (18), and Ravn (14) cite cases where the disease was more severe in the low areas of the field, and this accords with observations in southern Wisconsin. Drainage of such areas was recommended by Ravn (13) who cited cases where the disease had been checked by proper drainage alone. Anderson (1) reports a similar case of control by drainage, although in some instances he found the land became more subject to the disease after being drained. Halstead (7, 8) observed that watering increased the virulence of the disease during a dry season. Whitehead (20), Reed (18), Cunningham (6) and Ravn (15) noted that the disease was more severe after periods of wet weather. Humphrey (10) working in Massachusetts found the disease worse on the heavy and moist soils, while Reed (18) in Virginia notes that—

In soils which contain a large amount of organic matter (humus) and are warm and moist, the organisms find conditions very suitable for their growth.

Ravn, who, with his associates, conducted field experiments in Denmark for many years, gave more critical attention to the possible influence of soil moisture on the disease than any previous worker had. However, his conclusions were all

based upon observations with no direct experimental work. He frequently found a severe attack of the disease associated with heavy rainfall. In 1903 (16) he refers to a plot in which turnips were planted just previous to a heavy rain. The plants came up well, but had a sickly appearance from the start and were uniformly infected even where lime had been applied. In this same plot two years later he observed that the disease first appeared after a heavy rain of several days' duration and spread rapidly thereafter. In summarizing his observations on the influence of different seasonal conditions upon the occurrence of the disease (13) he was able to find no indication of a uniform severity or absence of the disease in any given area over a period of several years. He cites a number of cases where the disease was more severe on low ground, although he often attributes this to other causes in addition to excessive soil moisture. He reports (14) one plot which was planted on low ground where considerable water remained from fall to spring, although it was artificially drained. In this plot he observed that infection was most severe in the lower portions, which he attributed to excessive soil moisture and recent cultivation of turnips on the area. In another publication (13) he states that other things being equal, the disease is worst on the low parts of the field with wet, cold, and "sour" soil. He quotes 23 cases observed in 1905 and 1906, all of which show that the more moist the soil, the more malignant the infection. In a survey of plant diseases for 1916 and 1917 he again reports (17, p. 312) a case on low ground where infection was severe. Although this land had never been in cultivation before, only 6 of the 300 plants produced heads, all the others being destroyed by clubroot, probably in association with maggots. He also found that land which was poorly drained favored the development of the disease as did the low areas.

He refers to one plot (14) which had poor drainage where infection was unusually malignant at the close of August and continued to destroy the plants until harvest. The almost total destruction of the stand, notwithstanding the application of lime in the experiment, he considered as due to several cooperating circumstances, chief of which were probably deficiency of lime and defective drainage for he points out that on adjoining property having the same type of soil but which was well drained and marled there was little or no infection. Two other plots of this series at times showed severe infection in parts where drainage was not adequate, and he thought that this factor was partly responsible for the presence of the disease in this extreme form. He wrote (13) of a case where the disease had been increasing in severity for a number of years on an area which needed drainage. In 1901 the soil was drained, and the destruction due to clubroot was considerably reduced and had not increased since then in spite of the fact that cultivation of cabbage had been continued. He concluded that the moisture conditions of the soil were so important that all precautions against the disease are valueless when drainage is not perfect. He thus modified his recommendation of lime as a control for clubroot. After the long series of observations with Christensen and Harder (4) he recommends for control of this disease, a correction of acidity by liming. But where the soil does not need lime and the fungus is still present, he suspects defective drainage or some unfavorable physical condition of the soil and advises that these conditions be improved. He was convinced (16) that on well-drained arable land where there is sufficient calcium carbonate the introduction of the clubroot fungus will not cause any serious infection. As an explanation of the effect of weather conditions on the occurrence of clubroot, he seemed inclined to believe that an abundant precipitation at the time when the myxamoebae swarm in the soil will favor infection in a high degree, but he offers no proof of this. Conversely, he states that a warm, dry period in later stages of the disease may make it worse, since he frequently noticed infected turnip and rape plants wilting in

dry weather whereas in moist summers the disease often caused little damage, even when a large part of the plants were infected.

While Ravn’s observations seem clearly to justify his conclusions that, under the soil conditions prevailing in Danish fields, soil drainage, along with soil reaction, plays an important part, yet he gives no exact or experimental data upon which to base comparisons. He did not carry out any experiments to determine definitely the influence of water content apart from the other factors connected with poor drainage. While he follows the common habit of speaking of low or poorly drained soils as “cold,” we have no data on the possible influence of soil temperature as a factor in relation to clubroot infection or development. It seemed, therefore, worth while to attempt to segregate and define more clearly the possible influence of each soil temperature and soil moisture on this disease.

METHODS

Unless otherwise stated under the individual experiments, the method of procedure was in general as outlined below. In most of this work two distinct types of infested soil were used, both being in good physical condition and of high fertility. These will be designated throughout as A and B. Soil A was a clay loam obtained from a small home garden located at Chatham, N. J., where the disease had become serious and widespread. Soil B was a sandy loam, rich in organic matter, taken from a badly diseased portion of a commercial cabbage field near Kenosha, Wis. The water-holding capacity was determined for each soil by means of the usual 12-inch brass cylinder method. For comparison with these determinations the moisture equivalents were determined by means of the method described by Briggs and McLane (2), that is, by using a centrifuge with a radius of six inches and a speed of 2,400 revolutions per minute for 40 minutes. The wilting coefficient for each soil was calculated by means of the indirect method based on the moisture equivalent as described by Briggs and Shantz (3). The following is a summary of these determinations:

Water-holding capacity, moisture equivalent, and wilting equivalent of the clubroot infested soils

Soil	Water-holding capacity	Moisture equivalent	Wilting coefficient
	Per cent	Per cent	Per cent
A	45	24	13
B	62	34	19

To insure vigorous growth of plants the fertility of the soil was in each case increased by adding powdered potassium nitrate and dicalcium phosphate at the rate of 1 gm. of each chemical for every 3 kgm. of soil. Each lot was sifted through a 3-mm. sieve and the water content determined. Cans of equal weight were selected, and an equal weight of soil was put into each one so that when packed and planted the surface was about 1 inch below the top of the can. In order to make provision for water and aeration an inverted 3-inch flower pot connected with the surface by a glass tube was placed near the center of each can. When the soil was mixed, the water content was a little above that desired as the lowest moisture content to be maintained in the series. When a given series was repeated, the same soil was sifted, mixed, and replaced in the cans.

In all this work seedling cabbage plants were used. These were grown in sterilized soil and transplanted to 3-inch pots where they were allowed to grow for from three to eight weeks in a greenhouse maintained at a temperature

ranging between 15° and 20° C. The variety Copenhagen Market was used in the first series and Wisconsin Hollander in all subsequent series. Both varieties appeared equally susceptible to clubroot. Plants were selected as nearly equal in size and general vigor as possible for all cans of each series. The soil was carefully removed from the roots and all long roots were broken off to a length of 2 inches before transplanting into the diseased soil. Four plants were set in each can and the soil pressed firmly about the roots. The experiments were all conducted in the same greenhouse, and each series was arranged so that the light, air, temperature, and humidity would be as nearly equal as possible for all plants.

The cans used for the soil temperature series were 6 inches in diameter and 10 inches deep. In all the temperature series the soil in half the number of cans was adjusted to a high moisture content, and the remainder was kept at a low moisture content. The water in the wet soil was added after transplanting to avoid danger of "puddling" the soil around the roots at the time of transplanting. In the experiment in which the effect of soil moisture alone was studied, cans 5 inches by 5 inches were used. The procedure of preparing the soil, filling, and planting was essentially the same as that used for the temperature studies except that a circular glass plate 2 inches in diameter was used in place of the inverted pot. Water was added to these cans twice daily to avoid the necessity of adding very large amounts at one time.

The plants were grown in the cans of infested soil for periods of three to eight weeks, depending on the age when planted and on the rate of growth. In general, they were grown until the plants in some of the cans were severely injured by the disease and had become badly wilted. In removing the plants care was taken to preserve intact as many of the roots as possible. However, since the amount of naturally infested soil available was limited, it was necessary to sacrifice part of the smaller roots to avoid losing too much soil. Since in all cases infection was most severe on or near the stem or main roots, the loss of the smaller roots did not materially diminish the value of the results.

#### SOIL TEMPERATURE IN RELATION TO CLUBROOT INFECTION

For the soil temperature experiments the cans were placed in "Wisconsin soil temperature tanks" which have been described fully elsewhere (11). By this method the aerial parts of all plants in the same experimental series were exposed to like atmospheric conditions while the soil temperatures for the various plants were varied according to the needs of the experiment. The soil moisture was also varied with the experimental culture cans.

**SERIES I:** In the first series four tanks were maintained at 10°, 18°, 24°, and 30° C., respectively. Plants of commercial Copenhagen Market variety were used. Since the soil contained the organism of the yellows disease, *Fusarium conglutinans*, which is stimulated by high temperatures, most of the plants at the higher temperatures were badly injured by the latter disease. After three weeks they were removed and discarded since the presence of the two diseases rendered confusing results. However, since severe clubbing occurred on all plants grown at 18° C., while none were affected at 10°, the next series was maintained at temperatures of 9°, 12°, 15°, and 18° C. in order to determine the lowest temperature at which the disease would occur.

**SERIES II:** In this series two cans each of A and B soils were used for each temperature. The soil in one can was kept at a water content of approximately 35 per cent, and the soil in the other at 64 per cent of its water-holding capacity. After a period of one month the plants were removed and the roots examined. It was found that at the three higher temperatures uniform clubbing developed

on all plants in soil with the higher moisture content, but that only scattered infection occurred in soils with the lower moisture content. No case of clubbing was found in the soil kept at 9° C., even at the higher moisture.

**SERIES III:** Since the plants grown in soil maintained at 12° C. showed severe clubbing, only temperatures below 16° were included in later series with these soils. By doing this the soil was kept below the temperature range for infection by the *Fusarium*. Therefore, the third series was run in tanks maintained at 6°, 9°, 12°, and 15° C. As soil moisture appeared to be exerting a marked influence on the disease, the range in water content was increased. Soil A was maintained at 75 per cent and 30 per cent while soil B was maintained at 90 per cent and 30 per cent of the water-holding capacity. Since the plants were transplanted when considerably smaller than those used in previous experiments, they were allowed to grow for a longer period, approximately eight weeks. Upon examination of the roots it was found that in both soils with high moisture content all the plants were badly clubbed at 12° and 15° C. No cases of infection were found in either soil at 6° C. At 9° the plants grown in soil A showed no evidence of infection but each of the plants in the corresponding can of soil B in the same tank showed slight clubbing (Plate 3). Plate 2 shows the effect of these conditions on plants grown in clean soil. In no case of low soil moisture (either A or B) was there any evidence of clubbing at any temperature. Plate 1 shows one representative plant from each can of soil A at the higher moisture content.

**SERIES IV:** In order to determine the influence of higher temperatures, without confusion with yellows disease, four lots of soil were secured infested with *Plasmidiophora brassicae* but free from *Fusarium conglutinans*. One sample was obtained from the same garden as soil A and kept free from contamination with the *Fusarium* in the greenhouse. The other three were from Vermont, New York, and Minnesota, respectively,<sup>3</sup> from fields where clubroot was known to occur, but where yellows had not been observed. These soils were used for planting in tanks at 20°, 25°, 30°, and 35° C. The plants used in this series were larger than those in the previous temperature series and the period of growth in the infested soil was shorter.

Soil A was used at a water content of 30 and 75 per cent as in the lower temperature series. No clubbing occurred at any temperature in the soil maintained at 30 per cent of the water-holding capacity. In the soil maintained at 75 per cent of the water-holding capacity severe clubbing occurred at 20° C. but there appeared to be a reduction in the amount and severity of the disease as the temperature rose. Since there was a tendency for the soil to become dry more quickly at the higher temperatures due to the fact that the cans used for this series were smaller, and the plants were larger, it is possible that these conditions contributed to the results. The soils from Vermont, Minnesota, and New York were used in small cans (5 inches in diameter and 5 inches in depth) and kept at a moisture content of about 75 per cent of the water-holding capacity. The large plants, together with the limited amounts of water in these small volumes of soil and the fact that no insulation was used, made it so difficult to hold the moisture constant, especially at the higher temperature, that the results were not considered final. However, they were uniform in showing that infection was most severe at 20° C. and decreased as the temperature rose. Clubbing in all parts of the root system occurred at all these higher temperatures except 35°, in which case it occurred only on the main stem at the surface of the soil where its contact with the air may have resulted in a somewhat lower temperature.

<sup>3</sup> These were obtained through the courtesy of B. F. Lutman, Chas. Chupp, and E. C. Stakman, respectively.

SERIES V. In order that a comparison of the temperature influence over the whole range might be obtained in a single series, the lot of soil A which was free from *Fusarium conglutinans* was mixed with one-fourth its volume of quartz sand to make enough for 16 of the 6-inch cans. These cans were planted with plants more than two months old which had been somewhat checked in growth by being grown in small pots. The moisture was kept at 75 per cent of the water-holding capacity, and mineral wool insulation was used on the surface of the soil. Duplicate cans were placed in tanks kept at 6°, 9°, 12°, 15°, 20°, 25°, 30°, and 35° C. Wilting due to clubroot first occurred at 25°, and soon afterwards at 20° C. The growth of plants in these two temperatures was checked completely by the time the plants were removed. But plants at 15° and 30° seemingly were not checked in any way. Growth was very slight in the 35° tank. Failure to maintain the original low temperatures concluded the experiment before the disease had time to develop to the extremes which it might have attained in a more prolonged period. The plants were so large when transplanted that the relative growth of the tops did not indicate the actual effect of the disease and therefore were omitted from the photograph. The roots gave the best indication of the effect of the various temperatures upon development of the disease. Plate 4 shows two representative root systems taken from each temperature. The greatest development of clubs occurred at 20° and 25° C. There was also some clubbing at 15° and 30°, but none at the highest temperature 35° nor at the lower end of the series, 6°, 9°, and 12°. The results of this experiment are thus in accord with previous results.

The interpretation of the above results must take into account the effect of temperature on the host as well as on the organism. While temperature may limit infection by its direct effect upon the organism, it is obvious that with such a disease as clubroot its severity, as estimated by size of clubs, will depend to a great extent on the growth of the host immediately after infection. Tisdale (19) in his studies of the relation of soil temperature to the development of cabbage roots and tops has shown that the greatest root development occurs at about 20° C., with decided reduction above 25°; the present results are in accord with his findings. With regard to infection it appears from these results that clubroot infection will occur over nearly as wide a range as that in which growth of cabbage occurs. At the higher points the limits seem nearly to coincide. At the lower end of the range there seems to be a point (below 9° C.) at which infection does not occur and the host does grow very slowly. Between these two points (35° C. and 9° C.) there is a great difference in the severity of the disease. The largest clubs occurred at 20° C. which is close to the optimum temperature for root development. It may, therefore, be said that clubroot infection may occur at any temperature which is favorable for growth of cabbage and that the disease develops most rapidly and becomes most severe at about the optimum temperature for root development of this particular host plant.

In the individual series all plants were selected for uniformity of size and vigor; all were of the same age. However, there was a difference in age, size, and vigor between the different series. In general, it was observed that infection was less severe and development of clubs was slower in the plants that were older and less vigorous. Plants that had been temporarily checked before transplanting into the infested soil were slower in starting a vigorous growth in the new soil, and the disease was correspondingly delayed and less severe. This slow growth may explain some of the results of earlier attempts to produce the disease on old plants which led to the belief that infection occurred only on plants at the seedling stage. Kunkel (12) concluded that "old plants are almost as susceptible as young ones, provided they are growing." Although no plants

that could properly be called old were used in this work, it was noted that checking growth in general delayed development of the clubs. From the nature of the development of the organism within the host this dependence on host development seems probable.

### SOIL MOISTURE IN RELATION TO CLUBROOT INFECTION

In a preliminary test, using ordinary flower pots placed on a greenhouse bench and watered with a sprinkling can, there was a wide variation in amount of clubbing in the different pots, indicating the influence of soil moisture since other factors were equal for all pots.

Series I. In the more carefully conducted temperature series, however, watering was done with greater care, using 6-inch cans maintained at high and low soil moistures, as described in Series II and IV above. All of the plants grown in soil with the low water content remained entirely free from disease, while all of those grown in the same soil with the higher water content became diseased within the favorable temperature range. Plate 1 shows a representative plant chosen from each can of the higher moisture series in soil A. Plate 3 represents the corresponding series in soil B. An examination of Plates 2 and 3 will show some difference in plant development due to difference in water content in the cans of soil where they were grown. Undoubtedly the plants grow more vigorously in soil with the higher moisture, provided no clubbing occurs to check growth. However, it is seen that in soil containing insufficient moisture for disease development the plants could obtain enough water for considerable growth. All plants were of equal size when planted. Those grown at 6°, especially those in the low-moisture cans, made little growth and may be used for comparison to indicate the relative amount of growth in cans with different known moisture content.

Series II. To test further the influence of soil moisture on clubroot a series was prepared with cans containing an amount of water equal to 30, 45, 60, 75, and 105 per cent of the water-holding capacity of the soil in the can. Plants in the soil containing only 30 per cent of the water-holding capacity were not protected for a long enough period to enable them to become established; as a result they wilted and died. Those in the soil containing water to the extent of 45 per cent of its water-holding capacity had little difficulty in becoming established and grew well. The plants in the two cans with higher water content wilted badly during the day for some time before they were taken out. When they were removed, it was found that clubs had developed on all the plants except those in the 45-per cent can. The roots of the plants in the 105-per cent cans were almost completely decayed. Those in the 90-per cent can had become so soft that they could not be taken out of the soil without breaking, but the others were still firm. Plate 5 shows one plant from each can and illustrates well the dwarfing of the plants at the higher moisture content and the relative clubbing in each.

From the results of these investigations it seems that the disease develops only occasionally, if at all, in soils with a water supply less than half of the water-holding capacity. This limit is, no doubt, somewhat higher or lower in soils rich or poor in organic matter. With amounts of water above this minimum there is apparently a direct ratio between severity of the disease and increase in water content. At the higher moisture content it is probable that the severity of the disease is not limited to development of the clubs but is also due to a tendency of the clubbed roots to succumb more promptly consequent to their invasion by secondary organism. The optimum water content for development of cabbage plants is in general about 75 per cent of the water-holding capacity of the soil. This influence of excessive moisture in stimulating the clubbing

is apparently the same throughout the range of temperatures at which the disease is capable of development. It is noteworthy that whereas in the case of temperature the influence upon disease development was correlated with that upon host growth, by contrast in the case of moisture this does not obtain. In soils that are saturated with water growth of cabbages is practically stopped. The stimulating influence can be explained partly by the fact that excessive soil moisture favors the activities of the parasite and partly by the fact that it increases the damage due to secondary decay of the clubs. The disease may develop on the roots without severely checking the growth of the plant when the soil moisture is limited, but when the soil moisture is excessive the diseased roots are soon destroyed by secondary decay organisms and the plant dies.

The host tissue in tops and roots of plants grown in soil with this low water content appears to develop normally, although perhaps not quite as vigorously as that in soil with more available moisture. Some plants grown in infested soil at the low moisture content, on which the disease did not appear at the conclusion of the experiment, were grown in nutrient solutions for a period of three weeks longer after having the soil carefully washed from their roots. On these plants no galls developed whereas similar plants on which galls had started while growing in the wetter soil continued to develop the clubs after being placed in the nutrient solution. Therefore, it seems likely that the disease was avoided by preventing penetration of the organism rather than by checking the growth of the gall. After attempting to germinate spores on the surface of agar, Chupp (5) reports that "unless the spores were immersed in water there was no development." It is probable that failure of the disease to develop on plants grown in infested soils with low moisture content is due to insufficient moisture for spore germination. It has been observed that extended desiccation will check the viability of spores and prolonged periods of drought may destroy the organism in the soil.

## RELATION OF TEMPERATURE TO FIELD CONDITIONS

The indications are that under field conditions temperature is not a factor limiting the appearance of the disease, although it may exert an important influence on the severity of the disease if other factors are favorable.

Although the water content of the soil below which clubroot will not develop is insufficient for the most vigorous development of cabbage, it nevertheless provides moisture adequate for moderate growth. Under field conditions it is probable that there is frequently not sufficient soil moisture in the upper layer of soil to favor infection, and healthy plants at this time probably depend more on the deeper roots for their supply of water.

With these results in view it is interesting to note experiments conducted by Cunningham (6) in Vermont on the effect of "hilling up" cabbage plants as a method of controlling clubroot. He reports a method suggested by a Vermont grower, which consisted of hilling up the soil around the diseased plants to induce them to send out adventitious roots above the clubbed region. In explanation of the process, Cunningham notes that "The adventitious roots formed as a result of this practice on the stem above the diseased root are relatively disease free and function in the place of the affected roots which are practically useless." Further, he notes that "any procedure which induces club-free roots to form above the diseased roots greatly lessens the damage caused by the club." He conducted experiments during the course of two years in order to observe the effect of this procedure. "Decidedly beneficial results were secured in 1912, but in 1913 a decided injury was done." He, therefore, concludes that "hilling is most likely to prove helpful when the disease is most severe."



He also included observations on seasonal rainfall which aid in interpreting his results. He states that in 1913 the seasonal rainfall was only two-thirds of that of 1912, although both were below normal. The rainfall in 1912 was more evenly distributed and there was more cloudy weather. This was supposed to have stimulated the formation of adventitious roots. From this information we may infer that in 1912, the year in which clubroot was most severe, the soil was kept more moist by the even distribution of rainfall and by cloudy weather. Roots, therefore, probably became diseased in the flat soil which was moist enough to favor infection. The hilling process would provide drainage for the top layer of soil and probably reduced the moisture below the point where infection could occur. The adventitious roots developed in this hilled soil escaped infection because of the low moisture content of the soil immediately surrounding them. Enough moisture was present, however, so that the plants developed heads. The dry season of 1913 gave different results, probably because of excessive drying out of the soil beyond the point where roots could get enough moisture for growth. "The 1912 yield on some hilled plots was increased more than tenfold."

Similar development of adventitious roots was observed by the writer during the summer of 1920 in the Racine, Wis., district. Plants were badly clubbed in parts of some fields during the early growing period, and on hot days early in August these showed decided wilting. There was so little rainfall after July 1, that the soil became dry even in the lower areas. Adventitious roots, sent out from the stems above the clubbed areas, grew down into the soil beside the older, badly clubbed roots. These new roots did not become infected and in time were able to supply sufficient moisture to produce marketable heads; which were, of course, much below the average weight of heads produced on uninfected plants. It has been frequently observed in cabbage fields in various localities that when clubroot occurs regularly that it is usually most severe in the lower areas or depressions in a field. The continual favorable moisture conditions in such areas makes it possible for the organism to develop whenever wild or cultivated crucifers grow there and so the organism becomes abundant in such soil. On the other hand epidemics of the disease occur on higher and well drained soils which may be explained by the fact that the spores of this organism are known to be long lived and when the soil is once infested it only needs a season of abundant rainfall to make the disease severe even on this type of soils.

### SUMMARY AND CONCLUSIONS

It has long been recognized that clubroot is influenced by various environmental conditions. In recent years the work on this disease has been largely with the influence of soil acidity and the effect of liming. This has been especially true with studies in America, where liming has become the principal means of control. However, it has been frequently observed that the disease is not checked by liming the soil. These results indicate that there are factors other than soil acidity which largely influenced the severity of the disease, so experiments were conducted to try to determine what these factors might be. The work here reported shows the effect of soil temperature and of soil moisture as related to the occurrence of clubroot.

The trials were made by planting cabbage seedlings in naturally infested soil in the greenhouse. The air conditions were alike for all plants but the soil conditions were varied experimentally. The earlier trials aimed to determine the possible influence of different soil temperatures upon disease development, the later ones to determine the possible influence of different soil moistures.

In the temperature series using the Wisconsin tank method with soil temperatures, ranging by gradation from 6° to 35° C. clubroot developed throughout the entire range except at the extremes, that is, from 9° to 30° C. Within this range, 9° to 30° C., the effect of temperature upon the clubbing seemed directly correlated with its influence on the growth of the host tissues. The most severe injury to the plant occurs at a temperature somewhat above that for largest club production, wilting occurring sooner and dwarfing of the plant being more noticeable at 25 than at 20° C. The conclusion reached therefore is that temperature is probably not a direct limiting factor in clubroot development under field conditions.

On the other hand soil moisture was found to be an important factor. The trials included a soil-moisture range graduated from 30 per cent of the water-holding capacity of the soil to full saturation. The disease developed on plants grown in soil maintained at a moisture content of 60 per cent of the water-holding capacity and all higher moistures but did not develop in soil kept at 45 per cent or less of capacity. Injury to the plants increased with an increase in soil moisture above 60 per cent. Plants, however, are able to develop well (although not at their best capacity) with a soil moisture of only 45 per cent of capacity.

It is probable that failure of the disease to develop on plants grown in infested soils with low moisture content is due to insufficient moisture for spore germination. This may be due to an absence of enough water to provide a film around the spore, a condition which appears (5) to be necessary for spore germination. Prolonged periods of drought may even destroy the organism in the soil.

An important factor under conditions of excessive moisture is that of secondary decay. The disease may develop on the roots without severely checking the growth of the plant when the soil moisture is limited, but when the soil moisture is excessive the diseased roots are soon destroyed by secondary decay organisms and the plant dies.

Under natural field conditions a long period during which the soil is so dry that no clubbing, or only small clubs, would develop, may give the plants an opportunity to produce a good crop regardless of short wet periods. Such periods of low soil moisture occur during many seasons in most of our cabbage-growing sections and this no doubt explains the difference in severity of the disease in the same field in different seasons.

On comparing previous literature on clubroot with the evidence here presented as to the influence of soil temperature and soil moisture it seems probable that a consideration of these factors, and especially of soil moisture, may help to explain the conflicting results secured concerning the use of lime and the relation of soil acidity to the clubroot disease.

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## PLATE 1

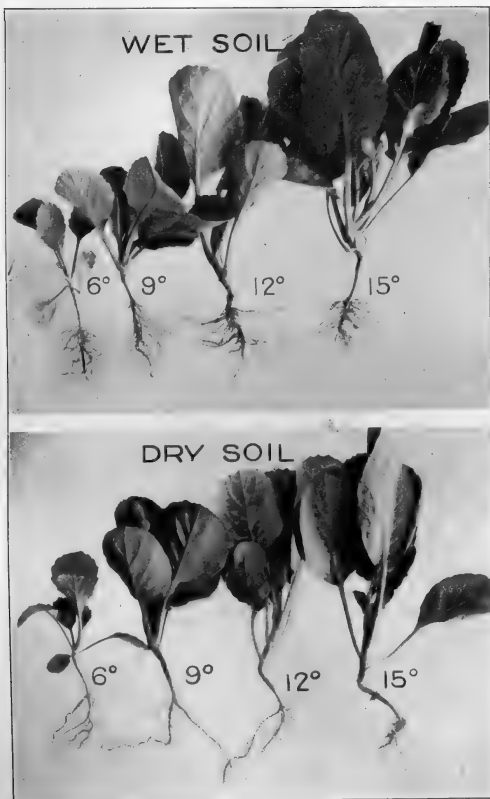
### INFLUENCE OF SOIL TEMPERATURE ON CLUBROOT OF CABBAGE

Note absence of disease at lower temperatures 6° and 9°, and the clubs at the higher temperatures, 12° and 15° C.

Plants grown eight weeks, at the soil temperatures indicated in a clay loam soil (soil A of the text, p. 552) uniformly infested with the clubroot organism. The air temperature for all alike was about 15° C. This soil was of a nature favorable to the development of clubroot and was kept uniformly at a moisture content found favorable for the development of the disease, that is 75 per cent of the water-holding capacity. The complete absence of clubroot in the two left-hand specimens must therefore be attributed solely to the inhibiting influence of soil temperature.

The relative size of these plants also deserves consideration. Note that normally at this range of soil temperatures with rising temperature there would occur a corresponding continued increase in the size of the seedlings (see Plate 2). Such normal increase in size is shown in the healthy seedling at 9° C., as compared with that at 6° C. The plant at 12° has evidently suffered some check as a result of the clubbing of its roots, and that at 15° has been much stunted because of the early and severe infection.





## PLATE 2

### INFLUENCE OF SOIL TEMPERATURE AND SOIL MOISTURE UPON THE NORMAL DEVELOPMENT OF CABBAGE PLANTS

Note the progressive increase in size corresponding to rising temperatures.

The plants were grown eight weeks, at the soil temperatures indicated, in a muck soil similar to soil B of text (page 552) but free from infection with the club-root organism. The air temperature for all alike was about 15° C. Plants in the upper group were grown in soil kept uniformly at high moisture content, that is 90 per cent of the water-holding capacity. Those below were grown in soil kept at a moisture content of 45 per cent of the water-holding capacity. When transplanted into this soil the plants were almost as large as those shown at 6° C. Very little growth occurred at that low soil temperature.

Note the somewhat larger plants grown in the soil with increased moisture.



### PLATE 3

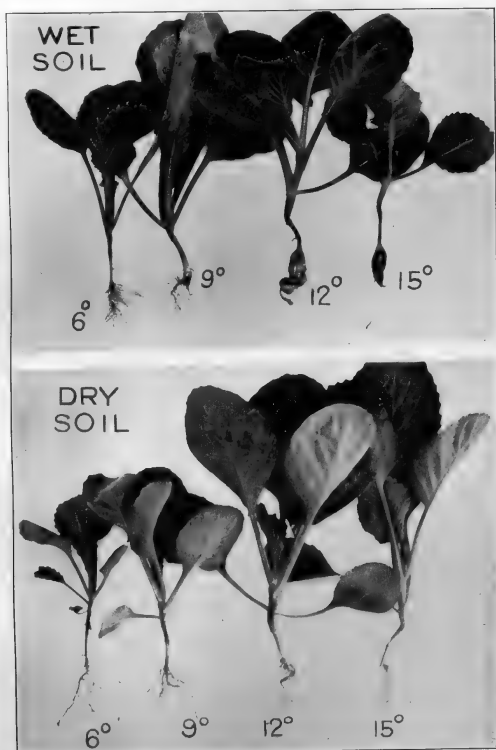
#### INFLUENCE OF SOIL TEMPERATURE AND SOIL MOISTURE UPON CLUBROOT OF CABBAGE

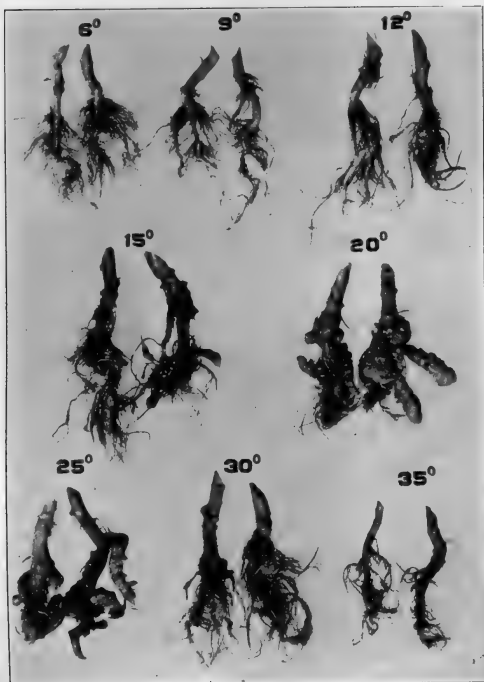
These plants correspond to those in Plate 2, but were grown in infested soil. Note that clubs occur only on roots grown in wet soil at 9°, 12°, and 15° C.

The plants were grown in soil taken from the same sample of sandy loam (soil B of text, p. 552) uniformly infested with the clubroot organism and of a nature favorable for the development of the disease. The air temperature for all alike was about 15° C.

Plants in the upper group were grown in soil kept uniformly at a high moisture content of 90 per cent of the water-holding capacity. Those below were grown in soil kept at a moisture content of 45 per cent of the water-holding capacity. The absence of clubbing in the roots from the dry soil at the three higher temperatures as compared with corresponding ones in the wet soil must therefore be due to the inhibiting influence of low soil moisture.

The influence of soil temperature on clubbing and on growth as shown in the upper group is similar to the series in soil A (Plate 1) with the exception of that grown at 9° C. In the latter case no clubbing occurred at 9°, but in this soil slight clubbing developed at 9° C.





#### PLATE 4

### INFLUENCE OF SOIL TEMPERATURE UPON CLUBROOT OF CABBAGE

Note absence of disease in the three lower (6°, 9°, 12° C.) and the highest temperature (35° C.).

Plants were grown 5 weeks, at the soil temperatures indicated, in a clay loam soil (soil A diluted with sand, see text p. 552 and 555) uniformly infested with the clubroot organism. The air temperature for all alike was about 20° C. This soil was of a nature favorable for the development of clubroot and was kept uniformly at a moisture content found favorable for the development of the disease, that is 75 per cent of the water-holding capacity.

The complete absence of clubroot in the three lower temperatures and in the highest temperature must therefore be attributed solely to the inhibiting influence of soil temperature.

Largest clubroot development at 20° C., with slightly smaller clubs at 25° C.

## PLATE 5

### INFLUENCE OF SOIL MOISTURE UPON CLUBROOT OF CABBAGE

Note absence of disease at the lower moisture, 45 per cent, and the extreme development at the highest moistures, 90 and 105 per cent.

Plants grown at like soil and air temperatures, which ranged from 12° to 18° C. Soil a sandy loam (soil B of the text, p. 552) uniformly infested with the clubroot organism and of a nature favorable for the development of clubroot. The amount of water constantly kept in the cans was equivalent to the per cent of water-holding capacity as indicated. The clubbed roots in the 75 per cent cans and above were partially decayed as a result of secondary decay organisms, so could not be removed without loss of parts of the roots that were diseased.



# LENGTH OF COTTON FIBER FROM BOLLS AT DIFFERENT HEIGHTS ON THE PLANT<sup>1</sup>

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The belief is current that in long-staple cottons fiber from the first picking is shorter than that from later pickings. This belief is probably well founded, for in 33 individual plant selections of the Yuma variety of Egyptian cotton, fiber from the second picking averaged one-sixteenth inch (1.6 mm.) longer than fiber from the first picking.<sup>2</sup>

The bulk of the fiber obtained from the first picking is taken from bolls on the lower fruiting branches, while the later pickings are furnished, in large part, by bolls situated higher on the plants. It is of interest, therefore, to determine the length of the fiber from bolls borne at different heights on the plant, in order to ascertain whether fiber length is correlated with the position of the boll. This paper gives the results of such determinations on the Pima variety of Egyptian cotton, grown under irrigation at the United States Field Station at Sacaton, Ariz.

Ten well-grown plants of Pima cotton were selected and a ripe boll was gathered from each successive fruiting branch on each individual. In order to determine whether these plants differed significantly in mean length of fiber, the fiber was measured on one seed from each boll collected on each individual.<sup>3</sup> The plant means as thus determined are given in Table I.

TABLE I.—*Mean fiber length of the 10 plants of Pima cotton used in determining the relation between height of fruiting branch and length of fiber*

Plant No.	Number of determinations	Mean length of fiber	Plant No.	Number of determinations	Mean length of fiber
		<i>Millimeters</i>			<i>Millimeters</i>
1.....	12	40.7±0.57	6.....	12	41.0±0.31
2.....	13	41.9±.66	7.....	12	43.1±.94
3.....	13	41.0±.31	8.....	11	41.9±.40
4.....	13	42.5±.75	9.....	12	40.7±.67
5.....	11	41.6±.41	10.....	11	41.9±.30

The individual plants show a range in mean fiber length amounting to 2.4 mm. but the difference between the plants having the longest and shortest fiber, respectively, is not significant, being only slightly more than twice its probable error. In computing the mean length of fiber for successive heights on the plant, it was therefore considered proper to take the bolls from all 10 plants as one array.

<sup>1</sup> Received for publication April 9, 1924.

<sup>2</sup> KEARNEY, T. H. FIBER FROM DIFFERENT PICKINGS OF EGYPTIAN COTTON. U. S. Dept. Agr., Bur Plant Indus. Circ. 110: 37-39. 1913.

<sup>3</sup> For a description of the method used in determining length of fiber see: KEARNEY, T. H. SEGREGATION AND CORRELATION OF CHARACTERS IN AN UPLAND-EGYPTIAN COTTON HYBRID. U. S. Dept. Agr. Bul. 1164, p. 10. 1923.

In order to smooth the curve and reduce the probable errors of the means, they were computed for each successive three fruiting branches, taken as one array. Fruiting branches in Pima cotton are rarely retained at nodes lower than No. 9 and the numbers of bolls which matured on fruiting branches higher than No. 32 were too small to give reliable data. The mean fiber lengths for the 8 groups of fruiting branches between nodes 9 and 32, inclusive, are stated in Table II and the data are presented graphically in figure 1.

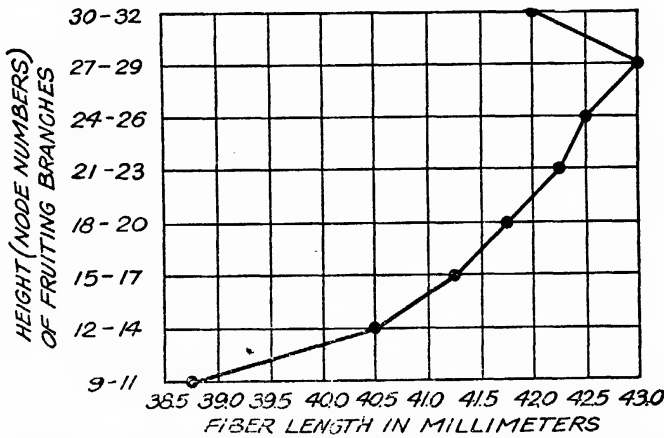


FIG. 1.—Average length in millimeters of Pima cotton fiber from bolls borne at successive heights on the plant, the heights being indicated by the numbers of the nodes of the main stalk at which the fruiting branches are borne.

TABLE II.—Mean length of Pima cotton fiber from bolls borne at successive heights on the plant, the heights being indicated by the numbers of the nodes of the main stalk at which the fruiting branches are borne

Node numbers of the fruiting branches	Number of determinations	Mean length of fiber	Node numbers of the fruiting branches	Number of determinations	Mean length of fiber
	<i>Bolls <sup>a</sup></i>	<i>Millimeters</i>		<i>Bolls <sup>a</sup></i>	<i>Millimeters</i>
9-11	10	38.75±0.85	21-23	10	42.20±0.45
12-14	19	40.46±.36	24-26	19	42.41±.25
15-17	11	41.27±.31	27-29	12	42.89±.40
18-20	20	41.75±.40	30-32	17	42.05±.40

<sup>a</sup> The absence of mature bolls on many of the fruiting branches at the time the collection was made accounts for the fact that the number of determinations is in all cases fewer than 30 (10 plants x 3 fruiting branches).

Although the differences in mean fiber length as between any two successive groups of fruiting branches, are in no case significant, there is a steady increase from the lowest to the next to the highest group. The highest group (nodes 30 to 32) shows a slight decrease in length of fiber as compared with the group just below (nodes 27 to 29).

The fiber in the bolls borne on fruiting branches at nodes 9 to 14, from which a large part of the first picking probably is derived, is decidedly shorter than the fiber produced higher on the plant. Comparing the lower half of the fruiting zone (nodes 9 to 20) with the upper half (nodes 21 to 32) the means for fiber length are  $40.75 \pm 0.28$  and  $42.37 \pm 0.19$ , respectively, the difference being  $1.62 \pm 0.34$ . This difference evidently is significant, being nearly 5 times its probable error.



It may be concluded, therefore, that bolls borne on the lower fruiting branches, constituting the so-called "bottom crop," produce shorter fiber than bolls that are situated higher on the plant.

Another series of determinations of fiber length was made on bolls from flowers which had opened in successive periods during July and August, 1921, the object having been to ascertain whether there is a consistent relation between the length of the fiber and the date of opening of the flower from which the boll developed, regardless of the height on the plant of the fruiting branch on which the boll was borne. In this case also fiber was measured on one seed from each boll.<sup>4</sup> The data obtained are given in Table III.

TABLE III.—Mean length of Pima cotton fiber in bolls from flowers which had opened during successive periods in 1921

Dates of flowering	Mean length of fiber	Dates of flowering	Mean length of fiber
	<i>Millimeters.</i>		<i>Millimeters</i>
July 22 to 24 .....	39.59±0.33	Aug. 14 and 15.....	41.09±0.32
July 27 to 30.....	41.90±.20	Aug. 23 and 26.....	41.72±.29
Aug. 8 to 10.....	41.36±.33		

The fiber from flowers opening during the first period (July 22 to 24) is significantly shorter than that from any of the later flowers but there are no significant differences in fiber length among any of the later periods. It is probable that the flowers which opened during the period July 22 to 24 were borne, for the most part, on low fruiting branches, while flowers which opened later may have been borne either on higher branches or on nodes farther out on the lower fruiting branches.<sup>5</sup>

While generalization from such limited data is unsafe, these results, as compared with those given in Table II, point to the conclusion that the length of fiber is affected less by the date of flowering than by the height on the plant of the fruiting branch on which the boll is borne.

<sup>4</sup> Through an oversight, no record was made of the numbers of determinations on which are based the means for each period.

<sup>5</sup> W. Lawrence Balls studied in Egypt the relation between date of flowering and length of fiber, tagging 20 flowers daily from July 7 to September 1. His data (BALLS, W. L. THE DEVELOPMENT AND PROPERTIES OF RAW COTTON, p. 198-203, Tables I-II; p. 90, fig. 4, London, 1915) show marked fluctuation during the flowering season, the minimum length of fiber having been reached in bolls from flowers opening during the 5-day period July 27-31 and the maximum length in bolls from flowers opening during the 5-day period August 9-13. The difference in fiber length as between the two periods amounted to 2.9 mm.



# "HAIRY NECK" WHEAT SEGREGATES FROM WHEAT-RYE HYBRIDS<sup>1</sup>

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Pollen from rye was successfully used to fertilize wheat flowers as early as 1875 by Wilson (14)<sup>2</sup> in Scotland. Subsequently many plant breeders, both in Europe and America, have made this cross and produced F<sub>1</sub> plants. The natural wheat-rye hybrid has been observed frequently in wheat plats, particularly at the Arlington Experiment Farm of the United States Department of Agriculture, Rosslyn, Va. (8) (see Pl. 1), and at Saratov, Russia (9). However, despite the many F<sub>1</sub> plants produced as the result of these artificial or natural cross-pollinations, few studies on the inheritance of specific morphological characters for other than the F<sub>1</sub> generation have been made and very few fixed segregates having any resemblance to rye have been reported. The plants described in this paper are believed, therefore, to be of peculiar interest in that they are like wheat, except for a single undoubted rye character, and in some cases have produced progeny all of which were like themselves.

## SELF-STERILITY IN WHEAT-RYE HYBRIDS

The cross between wheat and rye is intergeneric and the F<sub>1</sub> hybrid is either entirely sterile or only slightly fertile. As shown by Leighty (8), several experimenters have reported a small percentage of fertility in this generation. One natural hybrid found by Leighty had a fertility of 5 per cent, which is apparently unusually high. When the F<sub>1</sub> shows fertility and no precautions have been taken to prevent natural backcrossing, there is always the probability that the seeds present are due to the effect of wind-borne pollen of wheat or rye. The writers have no evidence that viable pollen is ever formed by the F<sub>1</sub> plants, and, furthermore, they have never observed opening of the anther sacs. Jesenko (4) found the F<sub>1</sub> wheat-rye hybrid self-sterile but slightly fertile with wheat pollen and very rarely so with pollen of rye. Investigations by the writers indicate that fertility in the F<sub>1</sub> nearly always is due to backcrossing with wheat pollen.

At Arlington Experiment Farm, during the crop seasons of 1922 and 1923, over 8,000 flowers of natural F<sub>1</sub> wheat-rye hybrids growing in the wheat plats were bagged with glassine bags before blooming and approximately 16,000 flowers were unprotected. The results, given in Table I, indicate that the F<sub>1</sub> was self-sterile, but slightly fertile when open-pollinated. Therefore, unless the pollination of the F<sub>1</sub> wheat-rye hybrid is controlled the source of pollen will be in doubt and inheritance can not be determined with accuracy.

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<sup>1</sup> Received for publication April 1, 1924.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 575-576.

TABLE I.—*Number of heads and flowers, bagged and not bagged, on F<sub>1</sub> wheat-rye hybrid plants growing in wheat plats and number and percentage of seed set on these heads*

Year.	Number of heads		Number of flowers		Number and percentage of seed set on heads		
	Bagged	Not bagged	Bagged	Not bagged	Bagged	Not bagged	
1922.....	142	240	6, 972	11, 791	a 1	Total 77	Per cent 0. 65
1923.....	34	69	1, 374	4, 772	0	32	. 67
Total.....	176	309	8, 346	16, 563	1	109	. 66

<sup>a</sup> The single seed obtained under bagged conditions is likely due to late or faulty bagging.

In this connection it should be explained that the rye varieties or selections are grown at the Arlington Experiment Farm in twentieth-acre plats, separated by several wheat plats in such a manner that the rye varieties are approximately 90 feet from one another. This tends to reduce somewhat the amount of natural crossing between the rye varieties, but furnishes abundant opportunities for natural crossing between the wheat and rye. Many natural hybrids between these have been found on this farm since 1914, the year in which the first one was discovered (6).

In 1918, 40 seeds were obtained from 19 natural F<sub>1</sub> wheat-rye hybrids found in the wheat plats at Arlington (8). The pollination of these plants was uncontrolled, and it is likely that the F<sub>2</sub> generation plants were natural sesqui-hybrids. The F<sub>2</sub> and F<sub>3</sub> of the wheat-rye hybrids were grown in the greenhouse, where natural crossing with wheat was again possible, though not to the degree existing under field conditions. The later generations were matured in the field rod-row nursery in order to test the yielding ability of the selections. The generation grown in 1922 consisted of 77 rod-rows, or approximately 9,400 plants. This generation was made up of about 63 strains, descended from 24 F<sub>3</sub> plants. These strains, in general, were fully as fertile in the F<sub>5</sub> generation as wheat grown under the same conditions. High fertility in these wheat-rye hybrids was first observed in certain plants of the F<sub>3</sub> generation.

The head type of the F<sub>5</sub> generation plants, grown in the 77 rod-rows, was for the most part identical with that of wheat, and only rarely was a plant found which differed from wheat to a degree that approached the F<sub>1</sub>. The majority of the plants could not be distinguished from common wheat. One undoubted rye character, however, was observed on eight plants which in other respects could be called wheat. This was the pubescent peduncle commonly associated with rye, but apparently unknown in wheat (Pl. 2).

### THE "HAIRY-NECK" CHARACTER

The peduncle is the upper portion of the stem (culm) and in the cereals is usually considered as being the uppermost internode at the apical node of which the head or spike is attached. The portion of the peduncle just below the head is often called the "neck," and when this is pubescent or covered with hairs, is here referred to as a "hairy neck."

The peduncle of rye is more slender than that of wheat, and its upper portion, the neck, for a distance of a fraction of an inch to three or more inches below the head, is usually covered with short hairs readily discernible to the naked eye. The lower segments of the rachis are also pubescent, but only the neck character is here considered. In some cases the hairiness, located around the apical node,

is limited to a few hairs which are difficult to distinguish without magnification. Rye plants with glabrous peduncles are sometimes found. The percentage of such plants is usually very small, but there apparently are varietal differences in regard to this factor despite the fact that the rye plant is almost entirely cross-pollinated. The necks of four typical rye heads are shown in Plate 2.

In Table II are given the data obtained on eight varieties of winter rye for the crop year 1922-23, in regard to the hairiness of the neck as seen by the naked eye. In five of the eight varieties, less than one per cent of glabrous-necked culms were found, while in von Rümker No. 2 there were 8 per cent. The total for all varieties was 1.9 per cent glabrous-necked culms. Gaines and Stevenson (3) report 66 per cent hairy-necked plants among 41 plants of Rosen rye examined at Pullman; Wash.

TABLE II.—*Number and per cent of glabrous-necked culms found in eight varieties of winter rye at Arlington Experiment Farm in 1923*

Variety	Number of culms examined	Glabrous-necked culms		Variety	Number of culms examined	Glabrous-necked culms	
		Number	Percentage			Number	Percentage
Von Rümker No. 2.	500	40	8.0	Winter.....	638	5	0.8
St. Johns.....	484	7	1.4	Abruzzes.....	643	4	.6
Rosen.....	1,363	45	3.3	Von Rümker No. 1.	700	4	.6
Virginia.....	490	3	.6	Total.....	6,587	128	1.9
Rimpau.....	480	4	.8				
Mexican.....	1,289	16	1.2				

The F<sub>1</sub> wheat-rye hybrid is approximately intermediate between the two parents in such characters as shape and density of head, number of spikelets, width and other characters of the empty glumes, and in general appearance (Pl. 1). The hairy-neck character of rye, however, is usually expressed in the F<sub>1</sub> and apparently is a dominant character in this generation. About 300 first generation wheat-rye hybrid plants, nearly all natural hybrids, growing at the Arlington Experiment Farm, have been examined with reference to this character. Approximately 80 per cent of these have had the hairy neck in the varying degrees as found in rye. The glabrous-necked plants in the F<sub>1</sub> may be due to the gametic constitution of the rye parents in these cases in respect to this character.

INVESTIGATIONS ON HAIRY NECK

Plants resembling wheat in most characters, but having the hairy neck, were observed in the F<sub>2</sub> and subsequent generations arising from the natural hybrids found in 1918. Investigations on this character were begun in 1920, when a cross was made between Purplestraw wheat and a wheat-like F<sub>3</sub> segregate of a wheat-rye hybrid. The pollen parent of this hybrid, the F<sub>3</sub> segregate, apparently carried the factor for hairy neck in heterozygous condition, for, from the 8 kernels resulting from this cross, 7 glabrous-necked plants were produced and only 1 with hairy neck. The progeny of this hairy-necked plant in 1922 consisted of 36 glabrous-necked plants and 2 with hairy necks. Data on the progeny of these 2 hairy-necked plants obtained in 1923, are given in Table III (A and B). In addition to these 2 plants, 8 other plants having the hairy neck were selected in 1922 from the F<sub>5</sub> progeny of the natural wheat-rye hybrids found in 1918. Data on the plants produced in 1923 from the seed of these 8 plants are given in Table III (C to K), and 3 of the heads, together with a head of Fulcaster wheat for comparison, are shown in Plate 3.

TABLE III.—*Total number of plants and numbers with hairy neck and glabrous neck in the progeny of each of 10 wheat-like plants with hairy necks selected in the progeny of wheat-rye hybrids, with data on certain head characters*

Selection number and head characters	Number of progeny plants			Head characters of progeny	
	Total	Hairy-necked			Glabrous-necked
		<i>Number</i>	<i>Per cent</i>		
Awnless, white-chaffed:					
A.....	92	13	14	79	(a)
B.....	100	16	16	84	(a)
C.....	40	40	100	0	(a)
D.....	28	9	32	19	(b)
E.....	66	5	8	61	(b)
F.....	59	13	22	46	(a)
Awned, white-chaffed:					
G.....	26	13	50	13	(b)
H.....	18	18	100	0	(a)
I.....	31	22	71	9	(b)
K.....	98	98	100	0	(c)

(a) All plants with the same head characters as the parent selection.

(b) One semiawned plant with red chaff included, all others with the same head characters as the parent selection.

(c) Eighty-four bearded, white-chaffed plants, 14 semiawned plants, all white-chaffed.

All 10 of the plants selected in 1922 had been fertilized naturally, no attempt being made to control pollination by bagging or isolation. These plants were fully fertile, with the exception of selection K, in which the fertility was about 75 per cent. The seed of these plants germinated well and about 75 per cent of the kernels produced plants the next year, which was fully equal to the performance of several pure lines of wheat sown in the same nursery.

The pubescence on the peduncles of the several selections made in 1922 varied somewhat in amount and extent. Several of the necks are shown in Plate 4, together with the necks of wheat and rye. The pubescence on selection I is very heavy, the hairs extending down the neck for 5 or more inches below the head, which is farther than has been observed on any rye plant examined. Selection K has a sparse pubescence, extending down the neck not more than an inch. The pubescence in the progenies of different selections in some cases was of much the same type as that of the parental selection, while in other cases different members of the progeny of a selected parent varied considerably among themselves as to pubescence.

In the progenies of the 10 selections, as shown in Table III, there were in all 247 plants with hairy necks and 311 with glabrous necks. Only 3 of these 10 selections, C, H, and K, produced plants all of which were hairy-necked. It is probable that these progenies are homozygous with respect to this character, but this is not regarded as a certainty. The expression of the character in later generations must be observed before it can be concluded that strains entirely homozygous in respect to the hairy-neck character have been established.

On the matter of the fixation of the hairy-neck character, Carman (1) who conducted some of the earliest and most extensive experiments on wheat-rye hybrids, reports as follows:

The Rural New Yorker No. 6 is one of the rye-wheat hybrids, though all appearance of rye has disappeared except that the culms just under the heads are now and again downy as in rye. This downiness of the stem is variable. We have tried by selection for many years to fix it without any approach to success. Of all our rye-wheat hybrids, the downy culm is permanent in but one, and that resembles rye in several other respects.

The progenies of the other 7 selections (A, B, D, E, F, G, and I) consisted of both hairy-necked and glabrous-necked plants, there being a total of 91 plants with hairy necks and 311 with glabrous necks, or very nearly a 1 to 3 ratio.

This is the ratio that would be expected if the hairy-neck character is recessive. But in the different progenies the ratios of hairy-necked to glabrous-necked plants varied from 1 to 12.2 in selection E to 2.4 to 1 in selection I. Furthermore, all of the parent plants had hairy necks. Therefore, the character does not appear to be recessive.

The hairy-neck character, however, has not been consistently dominant, as is evident from data given above. Nevertheless, at least a high degree of dominance occurs in the  $F_1$ . Additional data on the  $F_2$  generation of wheat-rye hybrids also are at hand. One lot of such plants grown by the writers in 1923 consisted of 21 with hairy necks and 8 with glabrous necks. These were descended from 24 natural hybrids, all with hairy necks, and all open-fertilized. Gaines and Stevenson (3) report that in the  $F_2$  they obtained 11 plants with hairy necks and 4 with glabrous necks.

### POSSIBILITY OF NATURAL CROSSING

It is known that natural crossing occurs to some extent in wheat-rye hybrids. The data presented in Table I indicate that fertility in the  $F_1$  is dependent on pollen from outside sources and not on self-pollination. The plants used in this study of the hairy-neck character were open-fertilized throughout the experiment, as were also all parental plants in preceding generations. In 1923, however, heads to be used in further studies were selfed by bagging. In this connection it should be noted that the percentage of fertility of 21 bagged (selfed) heads, excepting 2 in which the fertility for some reason was low, was 77 per cent, while the percentage of fertility of 21 unbagged (open-fertilized) heads, was 79 per cent. Some of the selected heads, both selfed and open-fertilized, showed higher fertility than Purplestraw wheat, which varied from 80 to 85 per cent, but others were somewhat less fertile.

The progenies of the selections listed in Table III do not disclose a large amount of natural crossing. All plants in 5 of the 10 progenies were identical with their respective parents in head characters. Four of the progenies each contained one individual that appeared from its head characters to be due to a natural cross on the parent selection, the off-type plant in each case differing from the parental selection in degree of awn development and in glume color.

Considerable natural crossing was evident in only one progeny (K), which consisted of 84 awned and 14 semiawned plants. These plants lacking full development of awns, in the progeny of an awned plant, indicate natural crossing with pollen from an awnless plant, inasmuch as the factor for presence of awns in wheat normally is incompletely recessive to the factor for their absence, which results in a semiawned plant in the  $F_1$ . In spite of this evidence of natural crossing all plants in selection K were hairy-necked. There is perhaps a slight possibility that the different head characters appearing in this progeny, likewise also in the other cases noted, are not evidence of natural crossing but indicate some unusual factorial composition of the gametes, resulting in abnormal segregation.

In addition to the differences in respect to awns, the progeny of selection K, though consisting entirely of hairy-necked individuals, also differed somewhat in head type, as is shown in Plate 5. All heads had white or yellow glumes, but one type had a lax, fusiform spike, while that of the other was more dense and oblong in shape. The width and shape of the shoulder of the outer glume, as well as the length of the beak, differed greatly, as may be seen from the figure.

In Plate 2 are shown the kernels obtained from one head each of Abruzzes rye, Fulcaster wheat, and the fusiform and oblong types of head present in selection K. Resemblance to rye may be seen in the seeds from the fusiform head. The

kernels are narrower, blunter at the base, and have longer and more sharply pointed germs than the kernels from the oblong head. The longer, narrower glume of the fusiform head no doubt influences the shape of the grain, but whether or not the glume character can be attributed to the  $F_1$  rye parent is open to question. According to Fruwirth (2, p. 183-184), a segregate somewhat similar to this fusiform type resulted from a wheat-rye hybrid made by Rimpau in 1888. It was largely wheat-like, but in comparison to wheat the head and glumes were longer, the latter closely pressed together, and the keel ciliated as in rye. This strain was grown for many years in an experimental way in Europe and remained fully constant in all respects, including a partial sterility exhibited by it.

### DISCUSSION AND SUMMARY

It is evident that the hairy neck found in wheat-like segregates of wheat-rye hybrids is a heritable character, but the data at hand are not sufficient to permit definite conclusions regarding the number of factors involved or the manner in which these factors are transmitted. Considerable irregularity in segregation is apparent and both dominant and recessive tendencies are observed. The reason for this is unknown, but it is suspected that it is related to probable irregularities in chromosome behavior.

According to Sakamura (11), *Triticum vulgare* has 21 and *Secale cereale* 7 haploid chromosomes. These numbers have been confirmed by Sax (12). The number of chromosomes in the  $F_1$  of the hybrid between these species has not been determined. Nakao (10), who made cytological studies of the  $F_1$  of a wheat-rye hybrid, states that "the chromosome number is not definite (17-23), being almost always more than the expected number, 16." <sup>3</sup> From our present knowledge of chromosome numbers in these species as given by Sakamura (11) and Sax (12), we would expect 28 chromosomes in the  $F_1$ , 21 from the *Triticum vulgare* parent, and 7 from the *Secale cereale*. This would be in accord with the observation of Kihara (5), who found 35 somatic chromosomes in the  $F_1$  of hybrids between different members of the "Emmer group" of wheat, which contain 14 gametic chromosomes, and members of the "Vulgare group" of wheat, which contain 21 gametic chromosomes. Sax (12) also observed 35 chromosomes in hybrids between these groups, while in an  $F_1$  hybrid between *T. monococcum* with 7 gametic chromosomes and *T. turgidum* with 14, the  $F_1$  had 21 somatic chromosomes. In the  $F_1$  hybrid between the "Emmer" and "Vulgare" groups, according to both Kihara and Sax, 14 pairs of chromosomes and 7 univalent chromosomes are found in the pollen mother cell. The 14 pairs, as stated by Sax, "divide as usual in meiosis while the 7 univalents lag behind but ultimately divide equationally in the first division, and pass at random, without dividing, to either pole in the homoeotypic division." As a result of these abnormalities, Sax estimates that 20 per cent of the pollen produced is obviously imperfect and a large percentage is nonfunctional. Considerable sterility is found in these hybrids between the groups of wheat. •

If such phenomena should occur in the wheat-rye hybrid there would be 7 bivalent and 14 univalent chromosomes in the  $F_1$  generation. With the higher number of univalents and the increased ratio of these to bivalents, abnormalities in chromosome behavior would be expected to occur in even greater degree than in the hybrids between different groups of wheat. Furthermore, with the gametes effecting the hybrid having come from plants belonging to different genera, incompatibility between the chromosomes contributed by the respective parents could hardly fail to exist. The results of Nakao (10) confirm this expecta-

<sup>3</sup> Nakao states: "In wheat, as described by Overton and Koernicke, and in rye, the reduced number of chromosomes is 8."



tion. In his studies on the nuclear divisions of the pollen mother cells of the wheat-rye hybrid he found degenerative phenomena in the pollen mother cell and in the pollen. "The degenerating phenomena of the pollen cells are observed at every stage of their development, e. g., in synapsis, spireme, the first division, the second division, etc." Jesenko (4) in studies of the pollen mother cell of the wheat-rye hybrid also found abnormalities which would seldom permit the formation of fertile pollen. These cells seldom divided into 4 parts, as is natural in wheat and rye, but frequently divided into 3, 5, 6, or even more different parts.

Pollen grains are formed in the  $F_1$  wheat-rye hybrid, but nearly all of them are shrunken and poorly developed, and lack the usual cell contents. A few are found now and then, however, that are better developed and that may be capable of growth. But anthers filled with such pollen have never been found, and, probably on account of the resulting lack of internal pressure, no anther has been observed to dehisce.  $F_1$  plants isolated or bagged have always been sterile. Pollinations made by artificially rupturing the anthers also have not been successful. Jesenko pollinated over 3,000 hybrid flowers with pollen forced out of the anthers but no flower set seed. The writers have made several hundred such pollinations with like result.

The  $F_1$  wheat-rye hybrid appears to be self-sterile, but seeds sometimes are set on such plants when open pollinated. In 1922, at the Arlington Experiment Farm, 11,791 wind-pollinated flowers of  $F_1$  wheat-rye hybrids growing in the wheat plats produced 6.5 seeds per thousand, and in 1923, under similar conditions, 4,772 hybrid flowers produced 6.7 seeds per thousand. The results for the two years agree so closely that it appears that heredity rather than environment is the factor determining seed formation. Leighty and Hutcheson (7) found, in 1919, that of the wheat flowers emasculated and exposed in the field at Arlington, 83.3 per cent set seed, a high seed formation for wheat heads. This indicates that an abundance of wheat pollen was disseminated by the wind to effect fertilization of exposed stigmas. It is well known also that rye pollen is widely disseminated by the wind. The flowers of the wheat-rye hybrid open at an average date intermediate between the average for wheat and rye, and, as is usual with small grain plants when fertilization has not occurred, remain open for a week or longer. Since the natural wheat-rye hybrids at Arlington grew in close proximity to both wheat and rye plants, there is little doubt that all their flowers were pollinated at some time or other with wind-borne pollen of both wheat and rye. The flowers thus pollinated produced on the average about 6.6 seeds per thousand. Jesenko (4) obtained 3 seeds per thousand pollinations when he backcrossed his  $F_1$  wheat-rye hybrid with wheat pollen (and one seed from almost 5,000 pollinations with rye). The difference in results obtained by the writers and by Jesenko probably indicates the greater effectiveness of natural pollination, and is in line with the results usually obtained in artificial crosses of wheat.

Although the  $F_1$  wheat-rye hybrid has produced no seed when bagged, but when open pollinated has shown 0.66 per cent of fertility, it cannot be definitely concluded that the female gamete is more fertile than the male. No cytological studies of the egg mother cell of the  $F_1$  wheat-rye hybrid have been made and nothing is known regarding the phenomena connected with development of the female gamete. Self-sterility, when a head is bagged, may be due to lack of development of sufficient pollen in the anther to cause dehiscence. Following artificial rupture of the anthers and self pollination, it may be due to the same causes that are responsible for the usually nearly complete self-sterility in rye itself. It is evident, however, that the stigmas of the  $F_1$  are brought into contact with viable pollen of wheat and rye more frequently than the pollen of the

F<sub>1</sub> is brought into contact with stigmas of any kind, and especially with ovules capable of being fertilized. Thus, the apparent greater fertility of the female gamete may be due merely to chance.

With 7 bivalent and 14 univalent chromosomes present in the F<sub>1</sub>, numerous recombinations at the time of gamete formation would be theoretically possible. It is probable that the gametes formed would contain 7 bivalents in every case and that nearly all of the gametes would contain, in addition, one or more of the univalents. Assuming random assortment, one gamete out of 16,384, on the average, would be expected to contain no univalent, and an equal number would contain all 14 univalents.

There is some basis for assuming that successful backcrosses on the F<sub>1</sub> wheat-rye hybrid with wheat and rye pollen are related in some way to the number of univalents present in the hybrid gamete, and that with a high number of univalents a successful backcross with wheat can result, while with none or possibly one univalent the rye backcross is possible. If it is assumed that a gamete with 12, 13, or 14 univalents is required for a successful backcross with wheat pollen the theory would explain the results obtained. The 12, 13, or 14 univalents present in the gamete would appear to counteract any incompatibility that may exist between wheat and rye chromosomes to a sufficient degree to allow the setting of seed.

Sakamura (11) found 42 somatic chromosomes in a fertile strain descended from a backcrossed wheat-rye hybrid. Kihara (5) confirmed this number in the fifth generation of this same lot of fertile hybrids. Two plants resulting from seed produced by an almost sterile plant in this same lot of wheat-rye hybrid descendants contained 38 and 42 somatic chromosomes, respectively.

Jesenko (4) observed a self-fertile plant with dense hairiness on the sheaths of the lower leaves in the progeny of an F<sub>1</sub> wheat-rye hybrid backcrossed with wheat. This character was not observed in either the wheat or rye parent. In the F<sub>3</sub> generation of this hairy-sheathed plant there were 31 hairy-sheathed individuals and 23 glabrous-sheathed, or approximately a 9 : 7 ratio. On the assumption that 2 factors are concerned in the inheritance, there should be 2 equal groups in the F<sub>4</sub>, segregating into 3 : 1 and 9 : 7 ratios, or a total of 3 hairy plants to 2 glabrous plants. The actual ratio obtained by Jesenko was 3 : 1.96. This is apparently an example of simple Mendelian inheritance in wheat-rye hybrids.

The unusual ratios obtained on the segregation for the hairy-neck character can not be explained satisfactorily, as they are based upon too few plants, and also upon plants in the ancestry of which natural crossing has occurred at least to a small extent. Further investigations may show that we are observing plants in which an excess of recessive factors, accumulated in the gametes through random assortment of wheat and rye chromosomes, results at times in a reversal of dominance, and at times in unusual ratios in inheritance. If this is the case it is possible that the three selections showing all hairy-necked individuals in their progeny are not fixed for this character, and that it may be impossible of fixation.

The F<sub>2</sub> and later generations of wheat-rye hybrids show greater fertility but less rye resemblance than the F<sub>1</sub>. As the F<sub>2</sub> is usually a sesqui-hybrid with the parentage (*T. vulgare* ♀ × *S. cereale* ♂) ♀ × *T. vulgare* ♂ (21 haploid chromosomes × 7 haploid chromosomes) × 21 haploid chromosomes, the rapid disappearance of the rye characters in generations later than the F<sub>1</sub> appears explainable. In the F<sub>1</sub> generation two complete sets of chromosomes are present, one from the wheat parent, the other from the rye. The possibility of obtaining, in the F<sub>1</sub>, an egg cell containing only 7 rye chromosomes or 21 wheat chromosomes, is extremely

small,<sup>4</sup> but if such occurred and fertilization were effected with a pollen grain from wheat or rye, respectively, the  $F_1$  type would be expected in the following generation. This has been seldom, if ever, realized. Individuals with certain rye characters and others showing little rye influence morphologically but exhibiting a high degree of sterility are to be found, however, in all generations so far grown, though the number of such individuals diminishes annually. The increasing resemblance to wheat and the increasing fertility is supposed to be accompanied by progressive change in the chromosome number and constitution in the direction of the wheat type. This would be comparable to the phenomena observed by Sax (13) in hybrids between wheat groups with different chromosome numbers.

The hairy-neck character dealt with in this paper is an example of a definite rye character present in individuals of the  $F_6$  generation which may be traced to the original rye parent, the parent with the lesser number of chromosomes. In other respects the selections possessing the hairy necks appear similar to wheat, and exhibit very little sterility. This is of interest and possibly of importance, inasmuch as the result obtained in interspecific wheat crosses, made with the aim of combining desirable characters of two species, have been disappointing for the most part, as the segregates reverted to the parental types, seldom showing recombinations of characters of both parents. The previously reported segregates of wheat-rye hybrids nearly always reverted rapidly to wheat, but it appears from the results reported above that it is possible to obtain plants combining certain characters of the wheat and rye parents.

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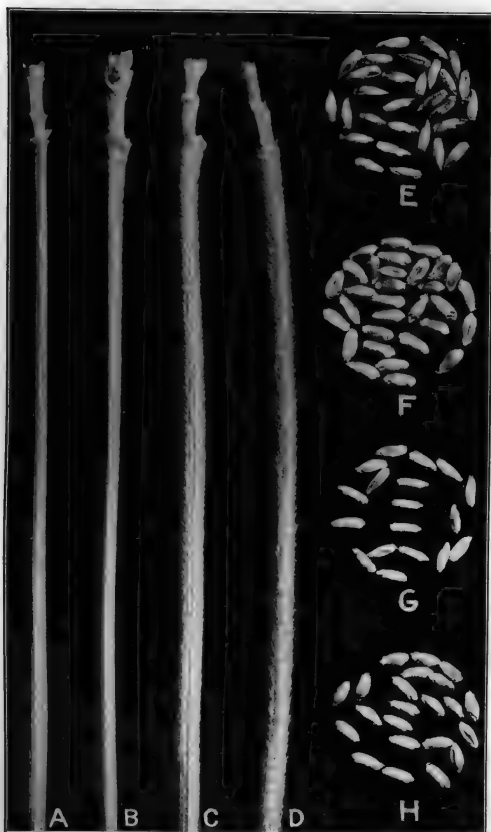
<sup>4</sup> Once each in over 2 million gametes.

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# PLATE 1

Heads of Purplestraw wheat (A), rye (D) and two natural  $F_1$  wheat-rye hybrids, one with pubescent or hairy neck (B), and one glabrous neck (C). (Natural size.)





## PLATE 2

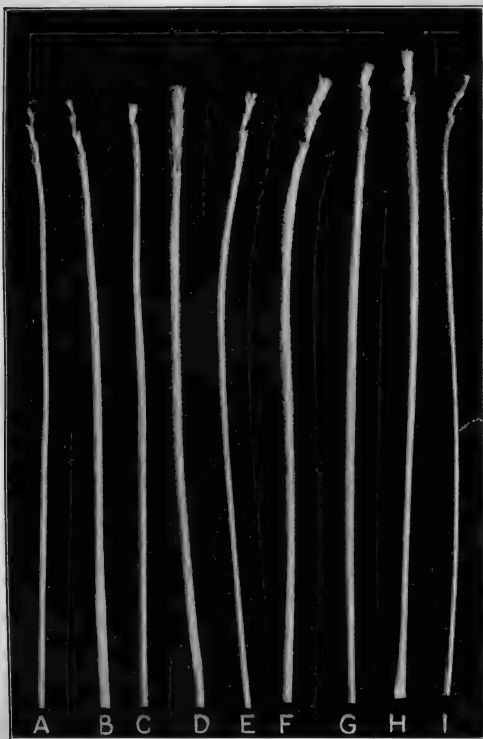
Necks (upper portions of peduncles) of rye culms: A, glabrous; B, sparsely pubescent; C and D, densely pubescent. (×3) Kernels of rye, wheat, and of two different types of head in the progeny of selection K (see Pl. 5). E, Abruzzes rye; F, Fulcaster wheat; G, fusiform head type (3) in selection K; H, oblong head type (4) in selection K. (Natural size.)



### PLATE 3

Heads of wheat and 3 wheat-like selections with hairy necks from the  $F_2$  progeny of natural wheat-rye hybrids: 1, selection K; 2, selection H; 3, Fulcaster wheat; 4, selection C. (Natural size.)





#### PLATE 4

Necks (upper portions of the peduncles) of the culms of wheat, rye, and selections from wheat-rye hybrids: 1, wheat; 2, selection K; 3, probable natural hybrid in progeny of selection 1; 6, selection D; 7, selection F; 8, selection C; 9, rye. (Natural size.)

## PLATE 5

Head types present in the  $F_2$  generation of wheat-rye hybrid plants with hairy necks: 1 and 2, probable natural hybrids found in progeny of selections I and K, respectively; 3 and 4, two types of heads in the progeny of selection K. Head No. 3 produced the rye-like kernels (G); and head No. 4 the wheat kernels (H) shown in Plate 2. (Natural size.)



## ASCARIS SENSITIZATION<sup>1</sup>

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It is a well established fact that *Ascaris lumbricoides*, the common intestinal roundworm of human beings and pigs, and other more or less closely related species parasitic in various animals, have toxic properties. This is clear not only from the nature of the symptoms in many cases of infestation which can be explained only on the basis of a toxic origin but also from the experience of many persons, mostly laboratory workers, who have had occasion to handle these parasites or who have in some way been brought into contact with them. Zoologists, for example, who have made extensive use of the horse *Ascaris* in cytological studies, have been frequent sufferers from its poisonous effects. Goldschmidt (1910) who was himself susceptible discovered by inquiry among zoologists of his acquaintance about 20 cases of similar susceptibility. (2).<sup>2</sup> Prior to Goldschmidt various writers, Bastian, Huber, Railliet, Linstow, and others, had reported personal experiences with the toxic effects of *Ascaris*, involving either the horse *Ascaris* or the human *Ascaris*. In fact susceptibility to *Ascaris* toxins is a very common condition among biologists who have been much exposed to contact with the parasite. Thus *Ascaris* sensitization is a sort of occupational disease. Its significance in relation to the toxic action of *Ascaris* in cases of infestation with the parasite remains to be determined.

The symptoms in persons who are sensitive to contact with *Ascaris* are of a kind that immediately suggest a similarity to the symptoms seen in cases of hay fever, asthma and other conditions grouped under the heading of foreign protein sensitization. Among the symptoms are irritation of the mucous membranes of the eyes, nose, and throat, lacrimation and edema of the eyes, facial edema, sneezing, coughing, swelling of the nasal mucosa, increased nasal and bronchial secretions, painful deglutition, urticaria, asthma, headache, fever, pruritus, tingling and burning sensations, swelling of the fingers, lassitude and weakness, sometimes amounting to prostration. In some cases the asthmatic attacks have persisted for weeks after exposure, but it is not certain in these cases that the possibility of subsequent exposures was entirely excluded. It has been commonly stated that the offending substance or substances are given off as emanations by the worm and thus are presumably volatile, but that they are actually volatile in the usual sense of the word has not been finally proved, although Weinberg and Julien (8) obtained a positive ophthalmic reaction in one out of 25 horses tested with the products of distillation of the body-cavity fluid of the horse ascarid, a result tending to support the prevalent belief in the presence of volatile substances in *Ascaris* that will produce symptoms in sensitive persons. Our own investigations thus far have failed to demonstrate a volatile substance that causes symptoms in human subjects sensitive to *Ascaris*, but we have not yet in our work taken up the question of those fractions of the worm that are insoluble in water.

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<sup>1</sup> Received for publication May 12, 1924.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 582.

The horse *Ascaris* appears to be more powerful in its effects than the human *Ascaris*, judging from the fact that more severe symptoms have been commonly reported among persons sensitive to the former than among those sensitive to the latter, but this may be related to the fact that the horse *Ascaris* has been in general more intensively worked with by zoologists than the human *Ascaris*, so that exposure has commonly been greater in the case of the former. Individuals may be sensitive to both species, as in the case of Goldschmidt, or sensitive to one and not to the other, as in the case of Bastian, in whom the horse *Ascaris* would cause severe asthmatic attacks but upon whom the human *Ascaris* had no effect. Whether an individual may be sensitive to the human *Ascaris* and not to the horse *Ascaris* has not been clearly established although we have been informed that one zoologist who was susceptible to the effects of the human *Ascaris*, experienced no discomfort after this susceptibility had been noticed in handling a large number of living specimens of the horse *Ascaris*. As noted by both Bastian and Goldschmidt the severity of the symptoms if originally mild may increase with repeated exposures, and in some cases the symptoms have become so severe as to force susceptible individuals to abandon further scientific work involving the use of the offending parasites.

The investigations that we have recently undertaken are the outcome of the discovery by one of us of his susceptibility to the toxic action of *Ascaris lumbricoides* on contact with the worm, (4) a discovery made about five years ago. At that time following the appearance of eye symptoms (irritation, lacrimation, and swelling of the eyelids) a cutaneous scratch test was made with some of the body fluid of the parasite, the results of which were positive. Later on one occasion the same individual experienced a severe general reaction as the result of a drop of *Ascaris* fluid falling unnoticed on a very small but fresh abrasion of the skin. This attack was characterized not only by a local wheal with surrounding erythema, but by extensive lymphatic involvement, which was evidenced by red streaks extending up the arm to the shoulder, swelling of the face, urticarial wheals over the entire body, very rapid heart, discomfort in the throat, and a sensation of warmth and breathlessness. The more alarming symptoms disappeared within a couple of hours, but the face and eyelids were still swollen the following day and the arm in the region of the local reaction was still swollen and reddened. More or less discomfort and various unpleasant symptoms have since been experienced under circumstances that involved direct or indirect exposure to *Ascaris*, although care has been taken to minimize the chances of close contact with the parasite so far as is possible in a parasitological laboratory.

In our recent investigations, which are still unfinished and of which a complete report can not now be given, certain observations have been made which appear to be of sufficient interest to warrant the presentation of a brief progress report.

About 20 persons, all white adults, have been tested for sensitiveness to *Ascaris lumbricoides* by means of a cutaneous scratch test. Among these there were 4 reactors, all of whom had been more or less exposed during periods of 5 to 25 years to contact with *Ascaris*. None of the reactors had a definite history of *Ascaris* infestation. Among the nonreactors were several who gave a history of having passed ascarids during childhood, and there were also several nonreactors who had at various times been considerably exposed to contact with *Ascaris* but had no recollection of unpleasant effects. Three of the reactors to the skin test had repeatedly experienced disagreeable symptoms on exposure to *Ascaris*; the fourth was previously unaware of his sensitiveness. In one of the cases the symptoms were asthmatic in type.

Different writers have reported that the characteristic odor of *Ascaris* may be detected on the breath of calves infested with *Ascaris vitulorum* and that the



meat of infested calves often smells so strongly of *Ascaris* as to render it unfit for food. In view of these reports the possibility naturally suggested itself that persons sensitive to *Ascaris* might also be similarly sensitive to pork from infested hogs if such meat contained toxic elements absorbed from the parasites infesting the animals. If it were found to be true that *Ascaris*-sensitive persons were sensitive to pork from infested hogs, the fact would be of great importance in its bearing on the question of pork sensitization, especially if at the same time such persons were insensitive to pork from noninfested hogs, inasmuch as a great deal of the pork that is eaten comes from *Ascaris*-infested hogs. Examination of about 2,500 hogs of various ages slaughtered in Chicago packing houses a few years ago showed an average of 41.1 per cent infested (3).

The following experiments were performed to determine whether pork from infested hogs contains a substance that will produce a skin reaction in persons sensitive to *Ascaris*. Aqueous, alcoholic, and ethereal extracts were made of pork from several infested hogs, and another similar series of extracts from a non-infested hog. Alcoholic and ethereal extracts were also made of fat from infested and noninfested hogs, respectively. None of these extracts gave a positive cutaneous scratch test in three *Ascaris*-sensitive individuals. Another experiment in which an aqueous extract of pork sausage was tested on six individuals, three of whom were sensitive to *Ascaris*, also gave negative results. The sausage used was a composite sample of sausage from several large meat-packing establishments. Any given lot of sausage manufactured in such establishments is likely to include trimmings from numerous hog carcasses, and in view of the frequent occurrence of *Ascaris* in swine the extract used in this experiment must have contained substances from the meat of a considerable number of infested hogs.

The results of these experiments, while not absolutely conclusive because of their negative character, indicate that the meat of *Ascaris*-infested hogs does not contain the substance that causes an urticarial skin reaction in *Ascaris*-sensitive individuals. In the light of this evidence there is no reason to suppose that *Ascaris* sensitization is involved in cases of pork sensitization.

Because of the writers' desire to learn something of the nature of the substance or substances in *Ascaris* that cause symptoms in susceptible persons, and, also, if possible, to isolate a substance that might appropriately be used to desensitize *Ascaris*-sensitive individuals, one of the writers undertook to separate various fractions of the constituent substances of the parasite. It was not considered advisable to attempt desensitization of human subjects with the crude substances of the worm inasmuch as certain pathological changes in the adrenals, thyroid, pituitary body, and pancreas of experimental animals have been recorded by other investigators following repeated injections of small quantities of the verminous material. Other investigators, also, notably Flury (1912), have reported the occurrence in *Ascaris* of a number of substances toxic to experimental animals. Flury (1) who has made the most extensive chemical and toxicological study of the parasite that has yet appeared has concluded that the toxic substances of *Ascaris* include aldehydes, free fatty acids, alcohols, esters, and two nitrogenous substances which are not protein in nature. He did not attempt to discover the substance which causes the urticarial skin reaction, and apparently was not familiar with this phenomenon. Shimamura and Fujii (1917) by fractionation of the watery extract from *Ascaris* separated a fraction which they termed "albumose-peptone" and to which they gave the name crude ascaron, applying the name ascaron to the inferred active principle, which they did not isolate (5). This ascaron they consider the active substance responsible for the toxicity of *Ascaris*, but their work throws little light upon the problem of the substance that causes the urticarial skin reaction.

Weinberg and Julien (6, 7, 8) have investigated the toxic action of the body-cavity fluid of the horse ascarid upon horses. The instillation of the fluid into the conjunctival sac was followed by a positive reaction in about two-thirds of 256 horses that were tested. This reaction which appears within a few minutes is characterized by edema of the eyelids, congestion of the conjunctiva, and lacrimation, occasionally accompanied by dyspnea, profuse sweating and diarrhea. Weinberg and Julien have concluded (8) that the toxicity of the fluid to sensitive horses is due to a number of active substances, inasmuch as they found the toxin to be thermostable, surviving exposure to a temperature of 120° C. for 20 minutes, filterable through a Chamberland filter, partially soluble in alcohol and in ether, and to contain volatile toxic constituents. The results that we have obtained in our investigations on human subjects with *Ascaris lumbricoides* are not entirely in accord with the conclusions expressed by Weinberg and Julien from the results of experiments with *Ascaris equorum* on horses. This discrepancy may be due not only to differences in the parasites and the experimental animals but also to the fact that Weinberg and Julien used body-cavity fluid of the worms in their tests while in our attempts to isolate an active substance we have been dealing with aqueous extracts of the worms and fractions of these extracts.

In our investigations swine ascarids (100 gm. in one lot and 1,774 gm. in another) after preliminary washing were ground up in a meat chopper, mixed with normal salt solution or 4 per cent ammonium sulphate solution and strained. The fluid portion was half saturated with ammonium sulphate and filtered. From the residue of this filtration a globulin fraction was obtained. The filtrate was precipitated by saturating with ammonium sulphate and after standing for 24 hours the albumens were collected by filtration. The filtrate was designated the protein-free filtrate for convenience although it still contained small amounts of proteins not precipitable by ammonium sulphate. It did not give the biuret reaction but gave a slight coagulum on heating. Three principal fractions were thus obtained from the original aqueous extract, a globulin fraction, an albumen fraction, and a so-called protein-free fraction. From the two latter various subsidiary fractions were prepared as shown in the list given below. Details of the chemical processes are reserved for a later report. The various fractions were tested on *Ascaris*-sensitive persons by means of the cutaneous reaction (scratch method). The results of these tests for each fraction are indicated by a plus or minus sign.

Fractions Nos. 1 to 4 were tested on three sensitive subjects, Nos. 5 to 9 on two, and Nos. 10 to 16 on one. In the test of fraction No. 8 the result was negative in the case of one subject and positive in the case of the other. The test was repeated in both cases with the same result, negative in one and positive in the other.

1. Globulin fraction.....	—
2. Albumen fraction.....	+
3. Protein-free filtrate (so-called).....	+
4. Protein-free filtrate heated just to boiling.....	+
5. Protein-free filtrate separated into approximately equal parts by distillation. Distillate.....	—
6. Same. Residue.....	—
7. Protein-free filtrate after air had been drawn through it for 55 hours.....	+
8. Protein-free filtrate treated with potassium permanganate.....	— (+)
9. Precipitate from acidified protein-free filtrate by Mayer's solution.....	+
10. Filtrate from acidified protein-free filtrate after treatment with Lloyd's reagent.....	—
11. Material recovered in weak alkali from Lloyd's reagent after action on acidified protein-free filtrate.....	+
12. Filtrate from acidified albumen fraction after treatment with Lloyd's reagent.....	—
13. Material recovered in weak alkali from Lloyd's reagent after action on acidified albumen fraction.....	—
14. Filtrate from albumen fraction after treatment with 50 per cent alcohol.....	+
15. Filtrate from albumen fraction heated 17 minutes in boiling water.....	+
16. Albumen fraction digested 36 hours with pepsin.....	—

From the results of these experiments, assuming for convenience of discussion that only one substance is involved, it is evident that the substance in aqueous extracts of *Ascaris lumbricoides* that causes the skin reaction in Ascaris-sensitive individuals is absent from the globulin fraction, present in the albumen fraction and present in the so-called protein-free filtrate, after the removal of the globulin and albumen fractions by precipitation with ammonium sulphate. It is weakened by oxidation with potassium permanganate so that it no longer produces a skin reaction in all Ascaris-sensitive persons. It is not volatile at temperatures between 20° and 100° C. It is thermolabile and is destroyed by exposure to a temperature of about 100° C. for less than an hour but may survive exposure to a temperature as high as 100° C. acting for a period of about 15 minutes. It is destroyed in the albumen fraction by digestion with pepsin. It is soluble in 50 per cent alcohol. From the acidified protein-free filtrate it is wholly adsorbed by Lloyd's reagent and wholly precipitated by Mayer's solution. It is also wholly adsorbed from the acidified albumen fraction by Lloyd's reagent but has not been recovered from the latter by subsequent treatment with weak alkali, a procedure which releases it from Lloyd's reagent after adsorption from the so-called protein-free filtrate. The question whether it is of protein nature has not yet been answered by our investigations.

In conclusion reference may be made to experiments which one of us has made with the body-cavity fluid and aqueous extracts of *Ascaris lumbricoides*, and extracts of the dried and powdered worms on various animals, by means of injections and local applications to the skin and eye. So far as concerns the local reactions produced in experimental animals which appear to be in a measure comparable to the urticarial reaction in human beings it is of interest to note that individuals sensitive to *Ascaris lumbricoides* may be found among both hogs (Pl. 1) and sheep. Local reactions have not been observed in guinea pigs or rabbits, which are relatively resistant to the toxic effects of Ascaris substance when injected subcutaneously, intraperitoneally, or intravenously, whereas sheep and hogs, particularly the former, are highly susceptible to the toxic action of Ascaris substance when injected parenterally. Some dogs will react to dog ascarids brought in contact with the conjunctiva, and Weinberg and Julien (6, 7, 8) as already noted have shown that horses commonly give an ophthalmic reaction to the body-cavity fluid of the horse ascarid.

Although the substances in *Ascaris lumbricoides* which cause local reactions in hogs and sheep may prove to be different from the substance that causes the local skin reaction in sensitive human subjects the fact that reactions very similar to that occurring in the human subject can be produced in lower animals as conveniently available as hogs and sheep promises to be helpful in the investigation of the question of Ascaris sensitization and utilization of these experimental animals for studies on Ascaris sensitization may lead to results having an important bearing on the general problem of sensitization of human beings to foreign substances.

#### SUMMARY

(1) Human beings are commonly sensitive to a substance contained in aqueous extracts of the nematode, *Ascaris lumbricoides*, an intestinal parasite of man and the pig.

(2) Sensitive individuals exhibit a positive skin reaction when this substance is applied to a scratch on the skin, similar to that exhibited in cutaneous tests for so-called foreign-protein sensitization.

(3) This substance is apparently absent from the meat of Ascaris-infested hogs and there is no reason to suppose that it is involved in cases of sensitization to pork.

(4) Cutaneous tests on sensitive human subjects made with various fractions of an aqueous extract of Ascaris material separated by chemical and physical

means have shown that the substance in this extract that causes the skin reaction is absent from the globulin fraction.

(5) It is present in the albumen fraction, and is also present in the filtrate after precipitation of the albumen and globulin fractions by ammonium sulphate.

(6) It is weakened by oxidation with potassium permanganate.

(7) It is not volatile at temperatures between 20° and 100°C.

(8) It is thermolabile and is destroyed by exposure to a temperature of about 100°C. for less than an hour, but may survive exposure to a temperature as high as 100°C. acting for a period of about 15 minutes.

(9) It is destroyed in the albumen fraction by digestion with pepsin.

(10) It is soluble in 50 per cent alcohol.

(11) From the filtrate obtained after precipitation of the globulin and albumen fractions with ammonium sulphate it is wholly adsorbed in the presence of acid by Lloyd's reagent, and wholly precipitated by Mayer's solution.

(12) It is also wholly adsorbed from the acidified albumen fraction by Lloyd's reagent but has not been recovered from the latter by subsequent treatment with weak alkali, a procedure which releases it from Lloyd's reagent after adsorption from the aqueous extract from which the albumen and globulin fractions have been removed by precipitation with ammonium sulphate.

(13) The question whether the substance in *Ascaris lumbricoides* that causes the skin reaction in sensitive human subjects is of protein nature has not been answered by the investigations herein reported.

(14) A substance or substances that cause local reactions in some hogs and some sheep, comparable to the reactions observed in human subjects, are present in *Ascaris lumbricoides*. The ophthalmic reactions observed in these animals are altogether similar to those heretofore observed by Weinberg and Julien following the instillation of the body-cavity fluid of *Ascaris equorum* into the eyes of horses.

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## PLATE 1

Ophthalmic reaction in a pig following the application of a few drops of *Ascaris* fluid to the conjunctiva. This animal also showed marked general symptoms in addition to the local eye reaction—vomiting, frothing at the mouth, frequent defecation, panting, and depression. The photograph was taken about 20 minutes after the application of the fluid.



# PHYSALOSPORA MALORUM ON CURRANT<sup>1</sup>

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A fungus having pycnidia and spores similar to those of *Sphaeropsis malorum* Berk. was reported on dead canes of cultivated currant (*Ribes* sp.) more than twenty years ago by Grossenbacher and Duggar.<sup>2</sup> These writers, however, made no suggestions as to its life history or specific identity. From the information now available it is apparent that the perfect stage of this organism is a *Physalospora*, and that the fungus must be regarded as identical with *P. malorum* (Berk.) Shear (*P. cydoniae* as used by Hesler) the cause of black rot of the apple (*Pyrus malus*). This fact is of pathological interest, for obviously the problem of disease control is complicated when a fungus of economic importance on one host occurs on various unrelated hosts. Morphological and cultural comparisons have proven also that the difference in the size and appearance of the fruiting structures of the fungus on the two hosts is due to differences in the substratum. The taxonomic significance of this is apparent, for once it is proven, as in the present case, that a readily observable morphological character of a fungus is not constant but varies according to the nature of the host upon which it is found, such a character can no longer be accepted as a basis of generic or specific segregation.<sup>3</sup>

## REVIEW OF EARLIER WORK

The suggestion that the apple blackrot fungus may occur on hosts other than those closely related to apple is of course not new. In 1899 Paddock<sup>4</sup> proved by inoculation that *Sphaeropsis* sp. from such unrelated hosts as *Diospyros virginiana* L., *Rhus typhina* L., *Celastrus scandens* L., and *Sambucus canadensis* L., will cause an apparently typical "blackrot" of apples. Paddock further maintained that the average size of the pycnosporangia varied in many cases according to the host on which they grew, yet the spores produced on apple fruits inoculated with cultures from these hosts were usually of the same size and character. Hesler<sup>5</sup> gives a long list of hosts, including *Ribes*, on which *Sphaeropsis malorum* has been collected and cites cross inoculation experiments with material from fourteen hosts.

As noted above, Grossenbacher and Duggar<sup>6</sup> report finding on currant canes a "Sphaeropsis-like fungus" which they refer to the genus *Haplosporella* in their early studies of currant cane blight.

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<sup>1</sup> GROSSENBACHER, J. G., and DUGGAR, B. M. A CONTRIBUTION TO THE LIFE-HISTORY, PARASITISM, AND BIOLOGY OF BOTRYOSPHERIA RIBIS. N. Y. State Agr. Exp. Sta. Tech. Bul. 18, p. 184. 1911.

<sup>2</sup> Many of the slides on which the morphological part of this paper is based were prepared by Rhoda Benham during the years 1919 and 1920, the rest by Ruth Colvin in 1923 and 1924. Much of the culture work was done by Marguerite Wilcox. The photomicrographs were made by Miss Colvin.

<sup>3</sup> PADDOCK, W. THE NEW YORK APPLE-TREE CANKER. N. Y. State Agr. Exp. Sta. Bul. 163, p. 194. 1899.

<sup>4</sup> HESLER, L. R. BLACKROT, LEAF SPOT, AND CANKER OF POMACEOUS FRUITS. N. Y. Cornell Agr. Exp. Sta. Bul. 379, p. 95-98. 1916.

<sup>5</sup> GROSSENBACHER, J. G., and DUGGAR, B. M. OP.CIT.

During the course of the present study of this disease which has extended over the last six years the writer has frequently found this Sphaeropsis on dead currant canes, often in close association with *B. ribis* or its form, *B. ribis chromogena*. It was not, however, until the summer of 1923 that mature perithecia of this fungus were found on currant and the identity of this fungus with *Physalospora malorum* established by morphological and culture studies.

#### ASSOCIATION OF THE PYCNIDIAL STAGE OF PHYSALOSPORA MALORUM WITH BOTRYOSPHERA RIBIS ON CURRANT CANES

Dead currant canes, whether killed by cane blight or not, frequently bear mature pycnidia of *Physalospora malorum*. Mature pycnidia of *P. malorum* often occur in close association with both *B. ribis* and *B. ribis chromogena*, the pycnidia of which as explained in an earlier paper<sup>7</sup> are of the Dothiorella type. On currant canes, moreover, the superficial appearance of the fruiting bodies of the fungi is somewhat similar. So closely, indeed, are the fungi associated and so similar their general appearance that during the early stages of our work pycnidia of the Sphaeropsis type frequently occurred in sections prepared for microscopic study of the currant cane blight fungus.

These considerations together with the fact that for several years only one type of ascospore was found on currant suggested the possibility that the "Sphaeropsis-like" pycnidia might be a stage in the life history of the Botryosphaeria or at least that the Sphaeropsis might be parasitic on currant. Inoculation experiments on currants with cultures made from single spores of the Sphaeropsis from currant and from other hosts gave, however, uniformly negative results. This was true even under conditions which gave striking infections when the true parasite, *Botryosphaeria ribis chromogena* was used. These results agree with those of Grossenbacher and Duggar.<sup>8</sup> Moreover, although several hundred single spore cultures of the fungi from currant have fruited, no connection could be established between the pycnidia of the Sphaeropsis type and either spore form of *B. ribis*. These facts made it appear highly improbable that the Sphaeropsis was a pycnidial form of the Botryosphaeria. Satisfactory proof of the distinctness of the two fungi was, however, not obtained until the summer of 1923 with the completion of the morphological and culture studies on *Physalospora* and *Botryosphaeria* on apple already reported<sup>9</sup> and the discovery of the ascogenous stage belonging to the Sphaeropsis on currant.

#### THE PERFECT STAGE OF THE SPHAEROPSIS ON CURRANT

Although the perfect stage of the cane blight fungus as well as the saprophyte *B. ribis*, has frequently been collected during the past six years and hundreds of single spore cultures have fruited, ascospores which produced pycnosporangia of the Sphaeropsis type in culture have been found only once on currant. This was at North Rochester, Mass., where, on June 27, 1923, a single currant cane covered with mature perithecia was found in a pile of old currant prunings. This material was at once recognized by Miss Wilcox, who was at that time carrying on culture studies of the currant cane blight fungus and related fungi at Woods Hole as different from *B. ribis* and very similar to *Physalospora malorum*. Thirty-seven

<sup>7</sup> SHEAR, C. L., STEVENS, N. E., and WILCOX, M. S. BOTRYOSPHERA AND PHYSALOSPORA ON CURRANT AND APPLE. Jour. Agr. Research 28: 589-598, illus. 1924.

<sup>8</sup> GROSSENBACHER, J. G., and DUGGAR, B. M. A CONTRIBUTION TO THE LIFE-HISTORY, PARASITISM, AND BIOLOGY OF BOTRYOSPHERA RIBIS. N. Y. State Agr. Exp. Sta. Tech. Bul. 18, p. 148. 1911.

<sup>9</sup> SHEAR, C. L., STEVENS, N. E., and WILCOX, M. S. BOTRYOSPHERA AND PHYSALOSPORA ON CURRANT AND APPLE Jour. Agr. Research 28: 589-598, illus. 1924.



single ascospore culture from this material all produced pycnospores indistinguishable from those of *Sphaeropsis malorum*. Subsequent investigation showed the fungus to be apparently identical with *Physalospora malorum*. The specimen here discussed has been deposited in the pathological collections of the Bureau of Plant Industry, United States Department of Agriculture.

COMPARISON OF THE PHYSALOSPORA FROM CURRANT AND FROM APPLE

The first characteristic which suggested that the material in question was related to *Physalospora* rather than *Botryosphaeria* was that the ascospores germinated by the single long unbranched germ tube characteristic of *Physalospora* and never, in the writer's experience, found in *B. ribis*. In cultural characters this fungus also agreed with cultures of *P. malorum* from apple on all the media tested and was readily distinguishable from *B. ribis* on such media as beef agar, potato agar, and corn meal in flasks.

The comparative size and shape of the spores from the two hosts is shown in Table I. The ascospores measured were taken direct from the host. The pycnospores, on the other hand, were grown in pure culture from single ascospores, as this seems to be the only method of making sure of the actual connection of the two spore forms. The close similarity in size and shape of the spores from the two hosts is apparent. This agreement is more marked in the case of the pycnospores, which were grown in pure culture under similar conditions, than in the case of the ascospores which were taken from the hosts. Whether this circumstance is significant and indicates either that the size of the ascospores is slightly modified by the host, as suggested by Paddock for the pycnospores, or that the form found on currant is actually slightly different from that on apple can not now be determined. This point could be settled only by the production of ascospores in quantity in pure cultures, or on the host under sterile conditions. Neither of these lines of attack is possible with our present knowledge.

TABLE I.—*Spores of Physalospora malorum*  
ARRANGED BY CLASSES ACCORDING TO LENGTH

	Length (microns)																			
	Total	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
Ascospores on apple.....	223	1	—	1	—	—	5	8	4	11	10	16	11	31	20	28	24	30	11	6
Ascospores on currant.....	146	—	—	1	—	4	8	9	15	9	30	15	12	21	4	6	6	3	2	—
Pycnospores in culture from ascospores on apple.....	100	—	1	5	1	18	17	14	20	11	9	—	3	1	—	—	—	—	—	—
Pycnospores in culture from ascospores on currant.....	100	1	1	13	5	14	13	16	11	15	5	1	3	1	—	1	—	—	—	—

ARRANGED BY CLASSES ACCORDING TO WIDTH

	Width (microns)										
	Total	6	7	8	9	10	11	12	13	14	15
Ascospores on apple.....	223	—	—	25	31	46	68	26	23	3	—
Ascospores on currant.....	146	1	—	2	14	23	41	33	22	9	1
Pycnospores in culture from ascospores on apple.....	100	—	—	—	4	8	36	25	17	8	2
Pycnospores in culture from ascospores on currant.....	100	—	—	—	6	8	36	30	11	5	3

TABLE I.—*Spores of Physalospora malorum*—Continued  
ARRANGED ACCORDING TO RATIO OF LENGTH TO WIDTH

	Ratio (microns)								
	Total	1.5	2	2.5	3	3.5	4	4.5	5
Ascospores on apple.....	223		6	46	77	58	21	11	4
Ascospores on currant.....	146	1	27	59	40	13	5	1	
Pycnospores in culture from ascospores on apple.....	100	3	42	51	4				
Pycnospores in culture from ascospores on currant.....	100	8	41	46	3	2			

RELATION OF SIZE AND STRUCTURE OF SPOROCARPS TO HOST

The only striking difference in the fungi on the two hosts is in the size and structure of the sporocarps and this the writer believes to be due to the effect of the substratum. Plate 1, A, shows in vertical section a typical mature perithecium of *Physalospora malorum* on apple, while Plate 1, B and C, show mature perithecia of *P. malorum* on currant. Usually on currant several perithecia are grouped together in a single sporocarp, as shown in Plate 1, B, and these are surrounded by and supported on a mass of stromatic tissue. Even when, as occasionally happens, a perithecium occurs singly on currant it will almost invariably be partly embedded in a mass of stromatic tissue.

A similar difference is found in the pycnidia. On apple the pycnidia usually occur singly and without any noticeable amount of stromatic tissue (Plate 1, D), whereas on currant the pycnidial cavities are usually grouped in large stromata (Plate 1, G, and Plate 2, A), or when they do occur singly are partly surrounded by stromatic tissue (Plate 1, F).

The presence or absence of a stroma has been considered of sufficient importance to separate species or even genera and families. That it can not be so considered in the case now under consideration is abundantly proven by the fact that the presence of a stroma in the fungus on currant is not a constant character. If a pure culture of the fungus from currant is permitted to fruit on a portion of sterile apple twig, the resulting pycnidia will mostly occur singly and will have little, if any, associated stromatic tissue. The same thing is true if such a culture fruits on any twig having a smooth bark with fine texture. Plate 1, E, for example, shows a typical pycnidium of the currant fungus grown in pure culture on a sterile portion of a black raspberry cane (*Rubus*). On the other hand, when the fungus from apple is grown on currant the condition is reversed, and the sporocarps are large and usually contain several spore cavities.

The experimental evidence that the size of the sporocarp is directly influenced by the host has reference at present merely to the pycnidial stage because we are not yet able to produce ascospores in quantity in culture. There is no reason to suppose, however, that the same relation would not hold for the perfect stage. In nature ascospores and pycnospores occur in similar sporocarps and on currant were found to occur in the same sporocarp.

Attention has already been called to the fact that the amount of stromatic tissue about pycnidia and perithecia of *Botryosphaeria ribis* is directly affected by the host upon which it grows.<sup>10</sup> In the case of this fungus as in *Physalospora* a larger ascocarp is formed when the fungus fruits on currant than when it fruits on twigs having a thinner and more closely knit bark. Whether this difference

<sup>10</sup> STEVENS, N. E., and JENKINS, A. E. OCCURRENCE OF THE CURRANT CANE BLIGHT FUNGUS ON OTHER HOSTS. Jour. Agr. Research 27: 837-844, illus. 1924.

is due to differences in the physical character of the bark or to the chemical composition of the food the twigs supply to the fungus must remain for further investigation.

That the change in amount of stromatic tissue and in arrangement of spore cavities is in fact due to the host is further indicated by the behavior of the closely related *Diplodia natalensis* Pole-Evans, which causes a serious rot of citrus fruits in Florida.

Plate 2, B, C, and D, show sections of mature pycnidia of *Diplodia natalensis* grown on sterile twigs of apple and currant from single spore cultures from grapefruit. It will be noted that on apple twigs the pycnidia are usually separate even when near together, whereas on currant twigs they tend to aggregate. The difference here must be due to the difference in substratum, since the cultures were all made at the same time by transfers from a single spore culture and during their growth on twigs were kept together in flasks on the same bench in a greenhouse for the same length of time. This would seem to indicate that in these fungi the size of the sporocarps and the amount of stromatic tissue associated with a single spore cavity is not a constant character.

### SUMMARY

The perfect stage of the Sphaeropsis common on dead currant canes has been demonstrated to be a *Physalospora*, which is apparently identical with *P. malorum*, the fungus causing blackrot of apples. The chief difference in the appearance of the fungus on the two hosts is in the size of the sporocarps, which on currant are large and usually contain several spore cavities, while on apple the sporocarps are much smaller and usually contain only one spore cavity. This difference in the size of the sporocarp is apparently due to the difference in the substratum. Pycnidia produced on sterile apple twigs from pure cultures of the currant fungus are small, while those produced on sterile currant twigs from pure cultures of the apple fungus are large and usually contain several spore cavities. A similar difference in the size of the sporocarps is found when pycnidia of *Diplodia natalensis* are produced in pure cultures on sterile twigs of apple and currant.

PLATE 1

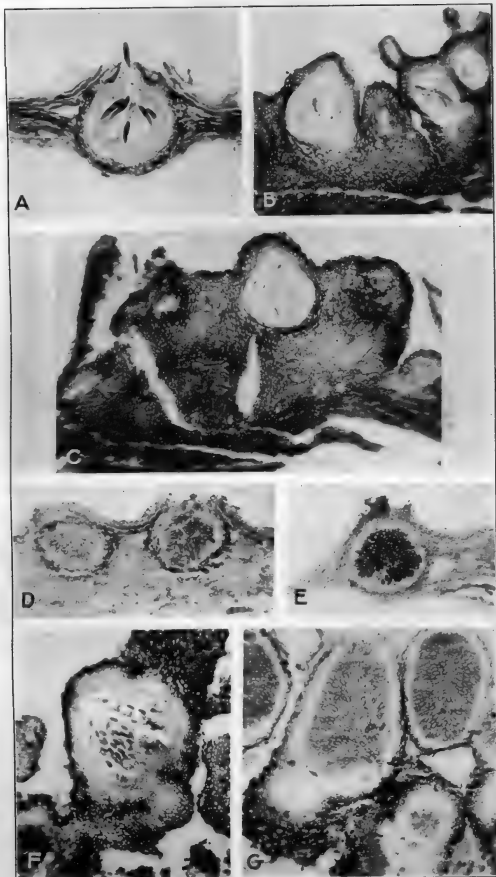
A.—Mature perithecium of *Physalospora malorum* on apple bark.

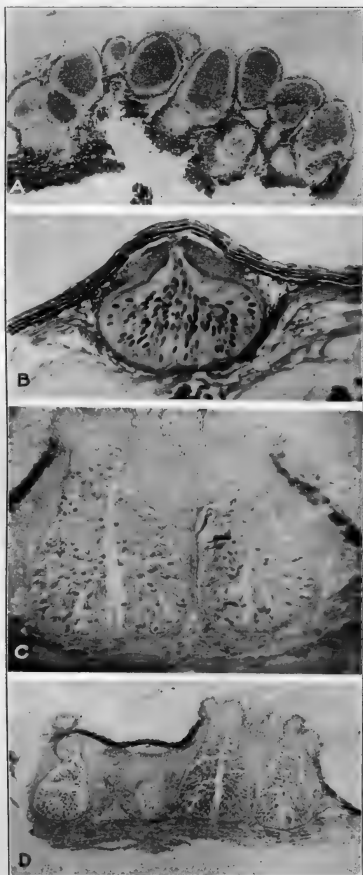
B, C.—Portions of sporocarps of *P. malorum* on currant bark; containing mature perithecia.

D.—Pycnidium of *P. malorum* on apple bark.

E.—Pycnidium of *P. malorum* on black raspberry from a pure culture from currant.

F, G.—Portions of sporocarps of *P. malorum* on currant containing mature pycnidia.





## PLATE 2

A.—Sporocarp of *P. malorum* on currant.  $\times 45$ .

B.—Sporocarp of *Diplodia natalensis* from grapefruit produced on apple twig in pure culture.  $\times 90$ .

C.—Portion of a sporocarp of *D. natalensis* from grapefruit produced on currant twig in pure culture.  $\times 90$ .

D.—Sporocarp of *D. natalensis* from grapefruit produced on currant twig in pure culture.  $\times 45$ .

# BOTRYOSPHERA AND PHYSALOSPORA ON CURRANT AND APPLE<sup>1</sup>

By C. L. SHEAR and NEIL E. STEVENS, *Pathologists*, and MARGUERITE S. WILCOX,  
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The economic importance of black rot of apple (*Pyrus malus*) and cane blight of currant (*Ribes rubrum*) seems to make advisable a statement of our present knowledge regarding their causal organisms even though our investigation of this group of fungi has not reached a point which warrants a detailed account of the synonymy of these species or of their life histories, genetic relationships, and distribution on the 26 hosts from which closely related fungi have already been collected and critically studied. The information at present available indicates that the ascogenous stages of these fungi are so similar as to suggest their very close relationship, and that both forms of *Botryosphaeria ribis*<sup>2</sup> occur on apple, on which host at least one form seems to be parasitic and capable of producing a fruit rot under some circumstances.

## REVIEW OF EARLIER WORK

The life history of the currant cane blight fungus was worked out by Grossenbacher and Duggar (2)<sup>3</sup> who also established its parasitism. To this fungus they gave the name *Botryosphaeria ribis*, distinguishing between the parasite and a purely saprophytic form which occurred commonly on currant, by the fact that the parasite developed a "purplish pink color" when grown on starch paste, while the saprophyte, which was morphologically identical with the parasite, developed no such color.

The literature on the black rot of apples and its causal organism is voluminous. Until 1913, however, it dealt almost exclusively with the pycnidial form of the fungus which was usually referred to as *Sphaeropsis malorum*. In December of that year, both Hesler (4) and Shear (10, 14, p. 107) published the results of pure culture studies which established the relationship of *Sphaeropsis malorum* to its ascogenous stage. This perfect stage Hesler referred tentatively to *Physo-lospora cydoniae* Arnaud, while Shear repeated his suggestion made three years earlier (9) in describing the life history of what is apparently the same species on grape, that it was *Melanops quercuum* (Schw.) Rehm forma *vitis* Sacc. or a variety of this species.

Three years later Shear and Beckwith (11) announced that pycnosporos of the *Sphaeropsis malorum* type had been produced in pure culture from single ascospores from a variety of hosts including apple, and that pycnosporos of the type of the currant cane blight fungus, which they refer to as a *Dothiorella*, have

<sup>1</sup> Received for publication Feb. 6, 1924.

<sup>2</sup> The name *Botryosphaeria* is used here for species congeneric with *Botryosphaeria ribis*, Grossenbacher and Duggar, as represented by specimens in Fungi Columbiani No. 3409 labeled *B. ribis achromogena*, Grossenbacher and Duggar. As thus defined the genus comprises part of the species included by Cesati and de Notaris (1) in their original description of this genus. This name is adopted instead of *Melanops* because of general usage. Fungi Columbiani No. 3408 represents *B. ribis chromogena* of this paper.

<sup>3</sup> Reference is made by number (italic) to "Literature cited," p. 598.



also been produced from single ascospores from apple, and other hosts. No suggestions are made as to the taxonomic status of these fungi, other than that they are closely related and that some of them have previously been referred to as forms of *Melanops quercuum*.

In 1919 Putterill (6) described a canker of apple trees in South Africa which was caused by a fungus closely resembling *Botryosphaeria ribis*. This fungus even possessed the chromogenesis (6, p. 264) described by Grossenbacher and Duggar for the currant parasite. The characters used by Putterill to distinguish his fungus, for which he proposes the name *Botryosphaeria mali*, are a difference in the width of the asci and in the size of the stromata. Recently Stevens and Jenkins (13) have demonstrated that the currant cane blight fungus occurs on horsechestnut, and certain varieties of rose, and that it is parasitic on the rose, causing cankers on the stem and sometimes killing whole canes.

### LIFE HISTORY STUDIES

**CULTURE METHODS.**—During the last two years the writers have had under observation over three thousand cultures of fungi belonging to this group, more than ninety per cent of which have fruited. In view of the difficulty experienced by some investigators in obtaining pycnospores of these fungi in pure cultures, a brief statement of the methods used may be of interest. It is certainly true that these fungi fruit but rarely in the arid and often superheated environment furnished by many laboratories. If, however, the cultures are kept in an ordinary greenhouse the temperature of which varies through a range from 50° to 70° F. or more, they will fruit abundantly on many agar media, on sterile twigs, and on cornmeal in flasks. Summer temperatures in the vicinity of Washington, D. C., are apparently too high to permit these fungi to fruit readily but good results have been obtained during the summer in unheated wooden buildings at Wareham and Woods Hole, Mass.

Our cultures were first transferred to the greenhouse in an endeavor to secure more variable temperatures, as a result of the observations of Stevens (12), recently emphasized by Harvey (3), that the temperature of bark out of doors often fluctuates with great rapidity. There is as yet, however, no certainty that the abundant fruiting is due to temperature range or fluctuation alone. It may as well be due to the greater humidity, or to a combination of temperature and humidity, or to some as yet unrecognized and incidental factor. It is sufficient for the purpose of the present investigation to be able to secure abundant pycnospore production with reasonable certainty in pure cultures made either from mycelial transfers or from ascospores or pycnospores.

**RESULTS.**—In the course of this culture work the writers have verified the life histories recorded above many times. Within the last two years alone pycnidia of the *Dothiorella* type have matured in pure culture from one hundred eighty single ascospores from currant and from seventy-seven single ascospores from apple. Mature pycnidia of the *Sphaeropsis* type have been produced in pure culture from ninety-five single ascospores from apple.

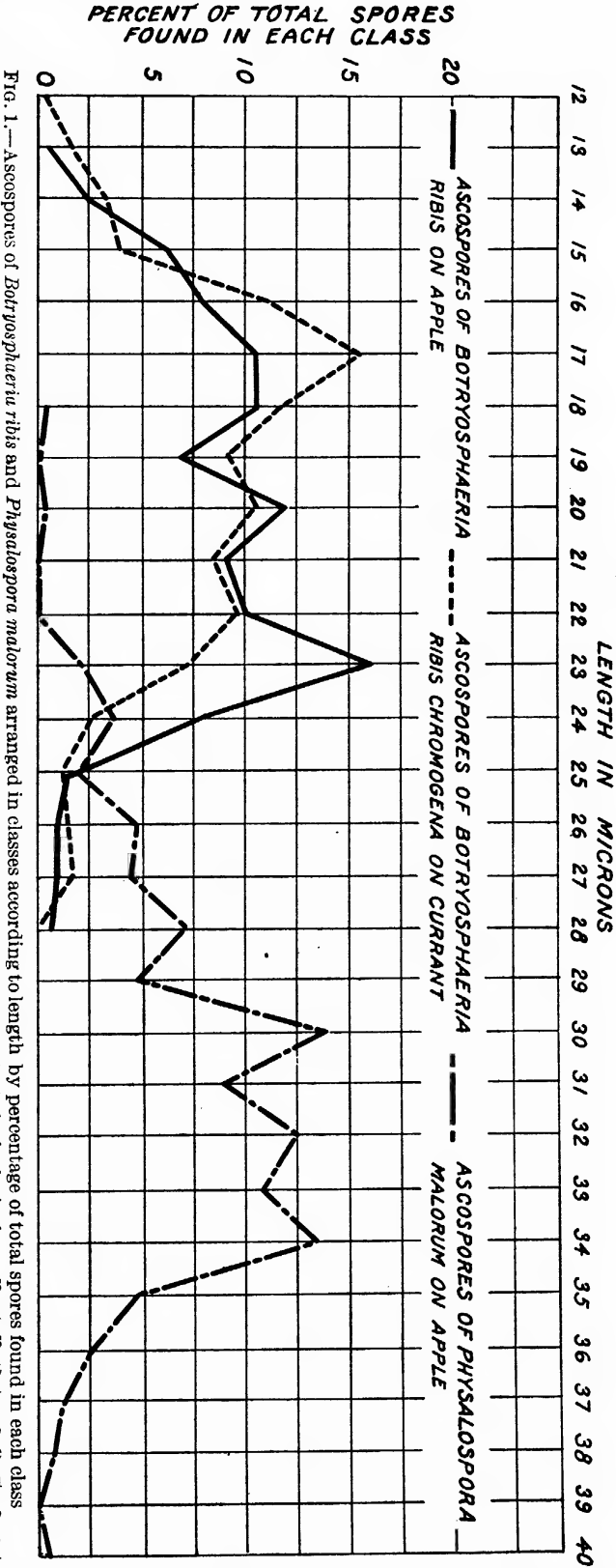
The name *Dothiorella* is here used to include such pycnidial forms as are congeneric with the macro-pycnidial stage of *Botryosphaeria ribis* and apparently also *Dothiorella gregaria* Sacc. Typical material is found in specimens 3407, 3408, and 3409, Fungi Columbiani. This is not *Dothiorella ribis* (Fuck.) Sacc. Number 3407 labeled "Macrophoma type" is a mere form of *Dothiorella* growing on young shoots and producing smaller and simpler pycnidia. The name *Macrophoma* has been most frequently applied to immature forms of *Diplodia* and *Sphaeropsis*, in which the spores are colorless. The name *Sphaeropsis* is here used to include pycnidial forms congeneric with the form usually called *S. malorum* on apple and whose ascogenous stage is *Physalospora*.

RELATIONSHIP OF THE FUNGI HAVING PYCNIDIA OF THE DOTHIORELLA TYPE

The collection and growth in pure culture of so large a quantity of material for a study of the life histories of the fungi, and especially the fact that a very large proportion of the cultures fruited in less than two months from the time they were made, has enabled us to make a more careful comparison of the fungi concerned than has hitherto been possible. Although the study has included material from a variety of hosts and from widely separated localities it is proposed to discuss here only the forms which are known to occur on apple and those described by Grossenbacher and Duggar on currant.

The writers' studies of the currant cane blight fungus and its saprophytic form have extended over a period of six years and have included the more important currant-producing regions of the United States. In general, the careful work of Grossenbacher and Duggar has been fully confirmed. The parasitism of the form which is characterized by the production of a bright pink color on starchy media has been confirmed by inoculation experiments in Virginia, both outdoors and in the greenhouse, as well as in Connecticut and Massachusetts. Inoculations with the non-chromogenic form have given uniformly negative results.

Continued collecting has, as might be expected, resulted in finding that the ascospores vary through a somewhat wider range than that given by Grossenbacher and Duggar, the great majority of the spores, however, fall within the limits set by them namely, ascospores, 16-23  $\mu$  by 5-7  $\mu$ , and pycnosporos 16-31 by 4.5-8  $\mu$ . Moreover, it is apparent that the size of the stromata varies very widely and is dependent to some extent on the thickness of the bark within which



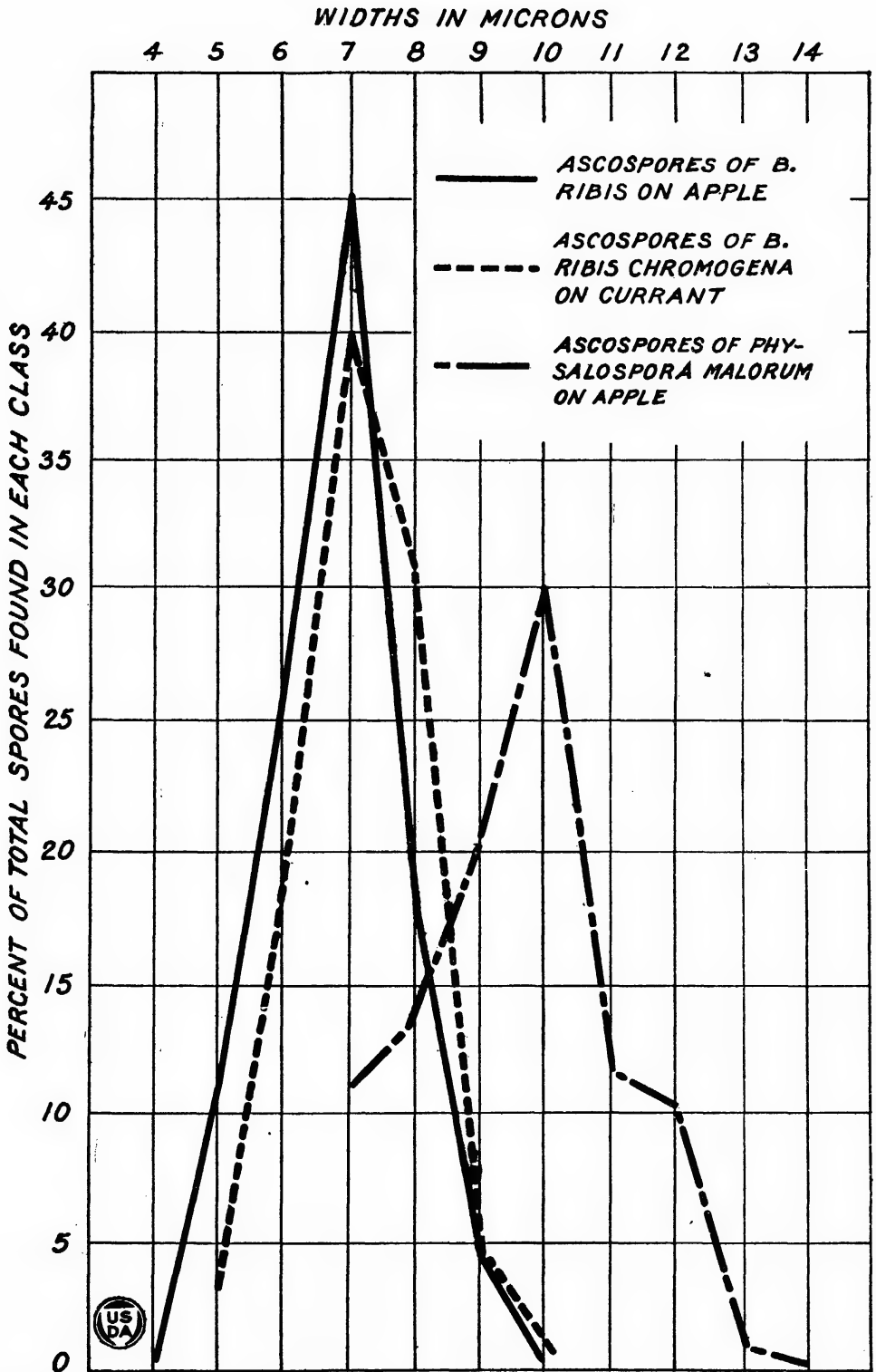


FIG. 2.—Ascospores of *Botryosphaeria ribis* and *Physalospora malorum* arranged in classes according to ratio of length to width, by percentage of total spores found in each class

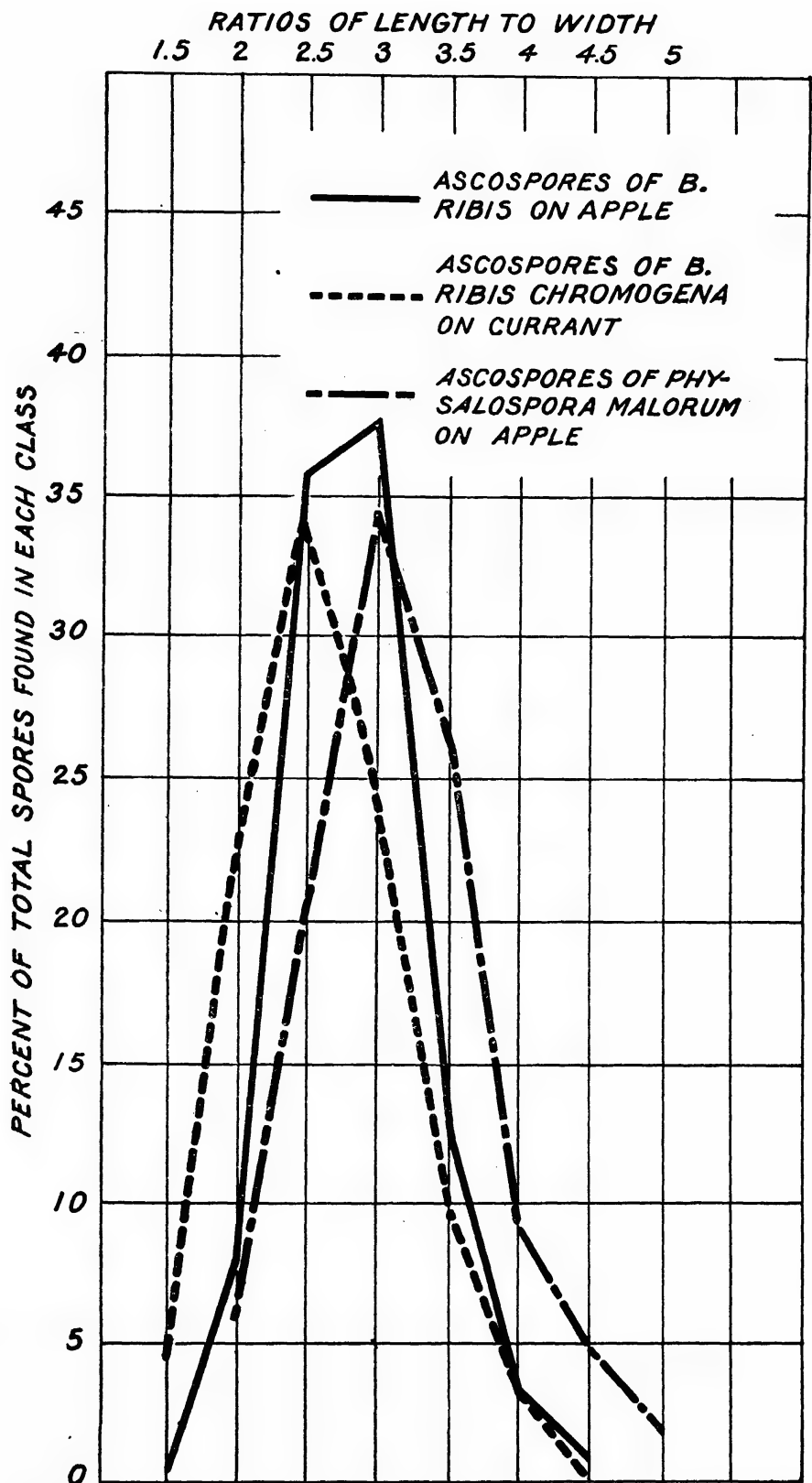


FIG. 3.—Ascospores of *Botryosphaeria ribis* and *Physalospora malorum* arranged in classes according to width, by percentage of total spores found in each class

they grow    Mature stromata of *B. ribis chromogena* have been observed on a single currant bush varying in size from 1 × 2–3 mm. on the smaller branches (Plate 1, C) to 3–3.5 × 5–8 mm. near the base of the older canes (Plate 1, D). As yet, the writers are unable to distinguish morphologically between the two forms of this species.

The most important contribution to our knowledge of *Botryosphaeria ribis* since that of Grossenbacher and Duggar has just been published (13). This is the proof that the parasitic variety which causes cane blight of currants, occurs in nature on at least two other hosts, horsechestnut (*Aesculus hypocastanum*) and several cultivated varieties of rose (*Rosa* sp.). Moreover, on rose this fungus is undoubtedly parasitic.

In view of the facts now in hand the writers are unable to escape the conclusion that the fungus found by Putterill on apple in South Africa is identical with *B. ribis chromogena* even though this fungus has not yet been reported on apple from the United States. Putterill himself points out the close resemblance of this fungus to that described by Grossenbacher and Duggar. In size and arrangement of perithecia and pycnidia his fungus agrees closely with theirs; the ascospores he finds to be 19.2–19.5 × 6.5–8 μ, and the pycnosporos 32.4 × 4.8 μ, which are well within the limits of *B. ribis*. (See Table I and figures 1, 2, and 3.) Moreover, (6, p. 264) his fungus possessed the chromogenesis which is characteristic of the parasite on currant.

TABLE I.—Ascospores arranged by classes according to length in microns

	Total number of spores	Class (in microns)																												
		13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	
Number of spores of <i>Botryosphaeria ribis chromogena</i> on currant in each class.....	226	5	7	9	25	35	27	21	24	19	22	16	6	3	3	4														
Number of spores of <i>Botryosphaeria ribis</i> on apple in each class.....	207	1	5	13	8	22	22	14	25	19	21	33	16	3	2	2	1													
Number of spores of <i>Physalospora malorum</i> on apple in each class..	223	--	--	--	--	--	1	--	1	--	--	5	8	4	11	10	16	11	31	20	28	24	30	11	6	3	2	--	1	

Ascospores arranged by class according to width in microns

	Total number of spores	Class (in microns)												
		4	5	6	7	8	9	10	11	12	13	14	15	
<i>Botryosphaeria ribis chromogena</i> on currant	226	---	8	44	91	70	11	2	---	---	---	---	---	
<i>Botryosphaeria ribis</i> on apple.....	207	1	11	54	94	37	10	---	---	---	---	---	---	
<i>Physalospora malorum</i> on apple.....	223	---	---	---	25	31	46	68	26	23	3	1	---	

Ascospores arranged by class according to ratios of length to width in microns

	Total number of spores	Class							
		1.5	2	2.5	3	3.5	4	4.5	5
<i>Botryosphaeria ribis chromogena</i> on currant ..	226	10	50	78	56	22	8		2
<i>Botryosphaeria ribis</i> on apple.....	207	1	17	74	78	26	7	2	2
<i>Physalospora malorum</i> on apple.....	223		6	46	77	58	21	11	4

The chief difference in the fungi, according to Putterill, lies in the fact that the asci of his form from apple are narrower than those measured by Grossenbacher and Duggar and the stromata of the apple fungus are usually about 0.5 mm. wide as contrasted with 2 mm. as the most common size on apple. Neither of these characters appears to the present writers to have significance. The asci are variable in width and are frequently found in material from currant no wider than those noted by Putterill. The stromata of this fungus, as already pointed out, vary in size with the thickness of the bark in which they grow. Mature perithecial stromata of *B. ribis* on rose are often 0.3 mm. or less in diameter, yet this fungus has been proven by inoculation experiments to be the same as that producing much larger stromata on currant.

That the character of the bark in which they are produced directly influences the size of the stromata in this species seems to be fully proven by their artificial culture on sterile twigs from different species of woody plants. Plate 1, A and B, show mature pycnidial stromata which developed on dormant apple and currant twigs from subcultures of *B. ribis* from currant. These cultures were started at the same time and kept near together in special culture flasks on a bench in a greenhouse from November 19, 1921, to February 22, 1922. A glance at the figures will show the relative size of the stromata on the two hosts. To be sure these are pycnidial stromata as the writers are not yet able to produce mature perithecia in quantity in pure culture. Under natural conditions, however, perithecia are often produced in the same stromata with pycnidia and on any given host the stromata bearing the two kinds of spores are of the same size.

Unfortunately the writers have not been able to secure cultures of the fungus from Africa for inoculation on currant, although Putterill has courteously tried to secure more living material for this work. Under date of January 25, 1921, he writes as follows:

"I have just returned from a visit to the infected trees at Vereeniging. Since my first visit there the trees have been more carefully treated, the diseased areas having been cut out and applications of coal tar made at regular intervals; I was unable to obtain fresh material for you."

In spite of the fact that inoculations can not be made, there seems to be no ground for considering the African fungus different from that which produces the cane blight of currants. Mere distance between the localities can not be considered a reason for considering the fungi different, especially when they occur on cultivated plants which are known to be shipped and carry their parasites long distances.

As to the possibility of this fungus being parasitic on apple in this country there is no evidence at present. Putterill's letter would suggest that it is sometimes not very virulent in South Africa since he was unable to find it in the orchard in which he had previously found it. That it can be made to grow on apple tissue is evident from the result of the inoculations made by Putterill on sound apples. Some years ago while checking up the results of shipping experiments on citrus for Rogers and Earle (8) the writers inoculated sound grapefruit with the currant cane blight fungus and produced a rot somewhat resembling the Diplodia stem end rot.

Since *B. ribis chromogena* is an active parasite on currant, Stevens and Jenkins were able to prove the identity of the material which they collected from horse-chestnuts and rose with the cane blight fungus by actual inoculation experiments. This type of proof is not possible in the case of the saprophytic or nearly saprophytic form. There is, however, no reason to consider the nonchromogenic *Botryosphaeria* which occurs on apple in this country as specifically distinct from the non-chromogenic saprophytic *Botryosphaeria* on currant, since no morphological differences have been found. (See Table I, text figures 1, 2, and 3, and Plate 2, A, B, C, and K-N).

The practice of describing as "new species" saprophytes or omnivorous parasites having practically identical morphological characters simply because they happen to be found on different hosts or in a distant locality, is to be deplored as it cumbers mycological literature with a mass of doubtful names and synonyms and greatly impedes the progress of mycological taxonomy. Real differences in essential morphological characters must be the foundation for specific segregation. What "real" and essential differences are can only be determined by careful and thorough study of a considerable amount of good material of the organisms in hand. Moreover when it has been shown by cross inoculations or studies of the fungi in pure culture that the characters upon which species have been segregated are such as result from host or cultural conditions and vary with a change of these conditions, they must be considered specifically identical.

## COMPARISON OF BOTRYOSPHERA RIBIS AND PHYSALOSPORA MALORUM

*Physalospora malorum* is here used for the fungus previously referred to as *Melanops quercuum* forma *vitis* by Shear (9) and *Physalospora cydoniae* Arnaud by Hesler (4), the pycnidial form of which is *Sphaeropsis malorum*. As there appear to be no good specimens of this species in published American exsiccata the writers will distribute what they regard as typical material of both stages to the principal large herbaria in the near future. The name *Physalospora malorum* is chosen as a combination of the best known names of the two stages of the fungus.

Both perithecia and pycnidia of *Botryosphaeria ribis* usually occur in a stroma, though single perithecia are often found and the size of the stromata varies, as has been pointed out above, with the host, and thickness of the bark. Hesler, in describing *Physalospora cydoniae*, the perfect stage of *Sphaeropsis malorum* (5), states that "the perithecia are usually scattered, standing separate from one another. Sometimes, however, from two to four fruit bodies are joined together, but no stroma has ever been observed." The writers have not yet made a sufficiently extended study and comparison of these characters to form an opinion as to their constancy and taxonomic value.

The paraphyses in both these species are very characteristic and have never, so far as we know, been correctly described or illustrated. They appear to consist of a tangled or anastomosing mass of septate, filamentous hyphae. In young perithecia the central portion is filled with a mass of pseudo-parenchyma. As the asci develops they push up into this mass, which becomes more or less filamentous at maturity, but separate paraphyses have never been observed.

The most striking difference in the perfect stages of the two organisms is the size of their ascospores. See text figures 1, 2, and 3, Table I, and Plate 2. As is evident from the Table and figures the longer spores of *B. ribis* equal in length the shorter ones of *Physalospora malorum* so that from a single spore it is not always possible to distinguish the two fungi. The great majority of ascospores of *P. malorum*, however, are longer than the largest yet measured from *B. ribis*. and in good preparations of mature ascospores the two species are easily distinguished on this basis. The same relation holds with regard to the width of the ascospores of the two fungi. The wider ascospores of *B. ribis* equal the narrower ascospores of *P. malorum* but as a whole the ascospores of *P. malorum* averaged about 3  $\mu$  wider. In shape as expressed by the ratio of length to width the ascospores of the two fungi are very similar indeed. See figure 3 and Table I.

The mode of liberation and ejection of ascospores has been observed in *Physalospora malorum* in fresh mature specimens mounted in water. The wall of the ascus ruptures transversely and the ascospores are ejected while still imbedded in a somewhat gelatinous matrix, having the same outline as the ascus and extending to the base where it appears to be attached, as illustrated in Plate 2, *F.* and *G.*

Micropynospores are found in culture and nature associated with the macropynospores of both the *Sphaeropsis* and *Dothiorella* types. They may occur in separate locules or be intermingled with the macropynospores. They are minute in size, about 2–3  $\mu$  long, oblong, and have never been observed to germinate. See Plate 2, figure N.

An interesting and apparently constant difference between the ascospores of the two fungi is found in their method of germination. Ascospores of *Botryosphaeria ribis*, whether from currant or from apple, characteristically develop two germ tubes which branch before they have reached more than 8 or 10 times (Pl. 2 O. and P.) the length of the spore. Even where only one germ tube is produced it usually branches while rather short. Ascospores of *Physalospora malorum*, on the other hand, usually develop only one germ tube which rarely if ever branches before it reaches a length equal to 50 or 60 times that of the spore itself. (Pl. 2, Q. and R.) So constant under the conditions of our work was this apparently trivial character that in the case of two hundred eighty-six ascospores of these two fungi in which the type of germination has been observed and the development of the pynospores in pure culture subsequently obtained, those ascospores which germinated by means of a single, long, unbranched germ tube have always produced pynospores of the *Sphaeropsis* type and those which germinated by means of the shorter branched germ tubes, usually two to a spore, have always produced pynospores of the *Dothiorella* type.

On many culture media early stages of the development of the two fungi appear much alike, especially when grown in dry air at temperatures above 22° C. They may be readily distinguished, however, on beef agar made according to the following formula:

Add 3 gm. of beef extract, 10 gm. peptone, and 5 gm. of sodium chlorid to one liter of distilled water. Steam one hour; titrate with hydrochloric acid and make up to plus 10, Fuller's scale. Add enough water to make one liter, and 1½ per cent shredded agar. Steam one hour. After cooling to 60° C. add the whites of two eggs well beaten—steam another hour, filter through cotton, tube and autoclave 20 minutes at 15 pounds pressure.

Cultures made on agar slants of this medium from mycelial transfers show after ten days a loose felt of short, rather fluffy mycelium on the surface of the medium. *Botryosphaeria ribis*, however, leaves the color of the medium unchanged (Baryta yellow) (?) whereas *Physalospora malorum* changes the medium to a dark color, between mummy brown and black.

Cultures on corn meal in 100 cc. Erlenmeyer flasks which have reached the stage of producing mature pynospores are readily distinguished by the surface character of the fungus growth. Fruiting or nearly mature cultures of *Botryosphaeria ribis* show numerous raised knob-like stromatic bodies usually 2 to 3 mm. wide and 3 to 4 mm. high in which the pycnidia are contained. (Pl. 1, E.)

Fruiting cultures of *Physalospora malorum* on corn meal in flasks on the other hand have a much more uniform surface without prominent regular elevations of any kind, the pycnidia being almost completely buried in the mycelial growth. (Pl. 1, F.)

If the various differences in stromata, size of ascospore, method of germination, cultural characters, and different pycnidial stages mentioned above are constant, they furnish a good basis for generic segregation. The two generic names which have commonly been applied to the two species on these hosts are here used for convenience and clearness.

#### SUMMARY

The life histories of *Botryosphaeria ribis*, causing cane blight of currant, and *Physalospora malorum*, causing black rot of apple, have been verified repeatedly by the development of the pynospores from single ascospores in pure culture.

The methods by which pynospores of these fungi were readily produced in pure culture are briefly described.



The differences between *Botryosphaeria* and *Physalospora*, as the names are used here, are the apparent difference in life histories, the first having *Dothiorella* as its pycnidial stage and the second *Sphaeropsis* as its pycnidial stage and the difference in ascospore sizes.

The ascogenous stages of *Botryosphaeria ribis* and *Physalospora malorum* may be distinguished by size of ascospore, method of germination of ascospores, and by certain cultural characters.

The fungus on apple in Africa described as *Botryosphaeria mali* by Putterill is apparently identical with the physiological variety of *Botryosphaeria ribis* G. and D. which the writers call *chromogena*.

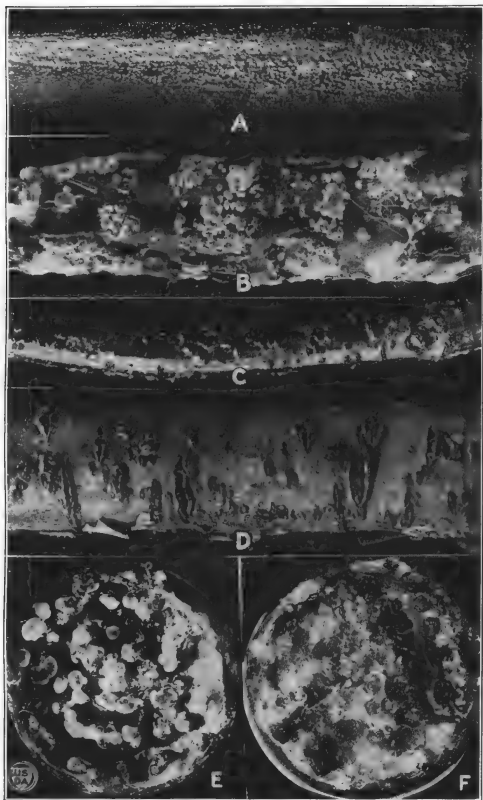
The fungus commonly found on apple in this country which has *Dothiorella* as its pycnidial stage and closely resembles that from Africa in morphology, but is nonchromogenic, is apparently identical with *Botryosphaeria ribis* G. and D.

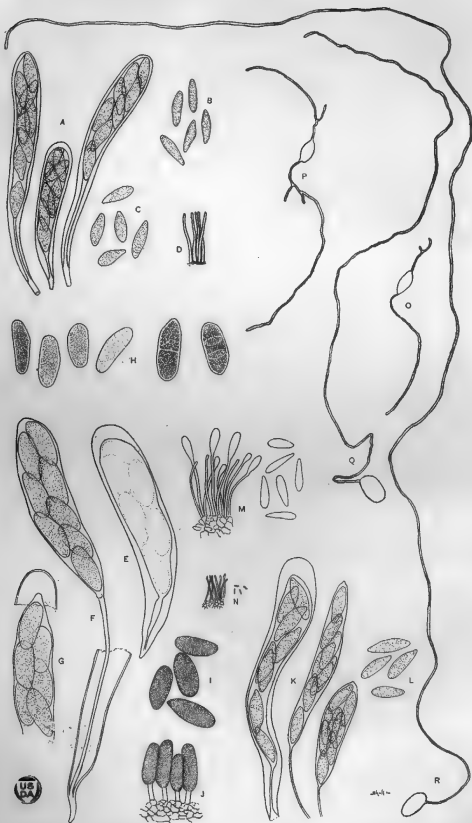
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PLATE 1

- A.—Mature pycnidial stromata of *Botryosphaeria ribis* grown in a flask on portion of sterile apple twig in greenhouse from November 19, 1921, to February 22, 1922.  $\times 2$ .
- B.—Mature pycnidial stromata of *Botryosphaeria ribis* grown in a flask on portion of sterile currant twig in greenhouse from November 19, 1921, to February 22, 1922.  $\times 2$ .
- C.—Mature pycnidial and perithecial stromata of *Botryosphaeria ribis chromogena* on small branch of dead currant bush. North Rochester, Mass., June, 1921.  $\times 2$ .
- D.—Mature pycnidial and perithecial stromata of *Botryosphaeria ribis chromogena* on trunk of same bush as that shown in C.  $\times 2$ .
- E.—Fruiting culture of *Botryosphaeria ribis* on corn meal in flask.
- F.—Fruiting culture of *Physalospora malorum* on corn meal in flask.





## PLATE 2

- A.—Three asci of *Botryosphaeria ribis* on gooseberry, from host.  
B.—Ascospores of *B. ribis* on gooseberry, from host.  
C.—Macropycnospores of *B. ribis*, grown in pure culture from single ascospores from gooseberry.  
D.—Sporophores of macropycnospores of *B. ribis* in culture.  
E.—Ascus of *Physalospora malorum*, on apple, from host.  
F.—Ascus of *P. malorum* showing mode of rupture and ejection of ascospores, from host.  
G.—Upper portion of a ruptured ascus of *P. malorum* with part of the ascospores, from host.  
H.—Ascospores of *P. malorum*, from host.  
I.—Macropycnospores of *P. malorum* from single ascospores from apple, cultures 2581 and 2660 from Vienna, Va., and Ambler, Pa.  
J.—Macropycnospores with sporophores from culture.  
K.—Three asci of *Botryosphaeria ribis* on apple, from specimen 2805 collected at Vienna, Va.  
L.—Ascospores of *B. ribis* on apple, from specimen 2805.  
M.—Macropycnospores of *B. ribis* on apple, some attached to sporophores, from culture 3106 A. from a single ascospore.  
N.—Micropycnospores and sporophores of *B. ribis* on apple, from culture 3106 A. Figures A to N.  $\times 420$ .  
O.—Germinating ascospore of *B. ribis* after 18 hours at a temperature of about  $12^{\circ}$  C.  
P.—Same spore held four hours after the stage shown in O—at a temperature of  $22^{\circ}$  C.  
Q.—Germinating ascospore of *Physalospora malorum* after 18 hours at a temperature of about  $12^{\circ}$  C.  
R.—Same spore held four hours after the stage shown in figure 1, at a temperature of  $22^{\circ}$  C. Figures O to P.  $\times 200$ .

# ISOLATION OF AN INHIBITORY SUBSTANCE FROM PLANTS<sup>1</sup>

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## INTRODUCTION

The isolation of lytic and inhibitory substances from animal sources by various investigators led the writers to a study of diseased plants in order to determine whether or not such substances were found in association with plant pathogens.

It seems needless to review the literature on bacteriophage since so many recent articles have included extended reviews. A number of investigators have found bacteriophage present in the intestines of man and animals in association with disease organisms. It is generally believed that bacteriophage develops under a diseased condition, adapts itself to the causative organism, and where a recovery results, it develops a high lytic power, causing a lysis of the causative organism.

A survey of the available literature failed to reveal any work of this nature on plant diseases; hence the present work.<sup>2</sup>

Soft rot of cabbage was selected for study, as it was easily obtained. A cabbage showing soft rot was placed in a large culture dish and allowed to decompose until a considerable quantity of liquid material had collected. At this point, loop dilution plates were made in order to isolate the rot-producing organisms. As the plates showed practically a pure culture, several colonies were fished and planted on agar slants. One of these cultures was used for all the work carried

TABLE I.—*The effect of the cabbage-rot filtrate on different organisms*<sup>a</sup>

Culture	Control	Amount of filtrate added		
		1 drop	2 drops	2 cc
<i>B. coli</i> .....	—	—	—	—
Do .....	—	—	—	—
<i>B. carotovorus</i> .....	—	—	—	—
Do .....	—	—	—	—
<i>B. melonis</i> .....	—	—	—	—
Do .....	—	—	—	—
Cabbage-rot organism .....	—	±	±	±
Do .....	—	±	±	±

<sup>a</sup> The following symbols are used in the tables: —, very strong turbidity; ±, slightly less turbidity; +, cloudy; ++, less cloudy; +++, nearly clear; +++++, clear.

out. The collected liquid was filtered through a filter paper, previously impregnated with diatomaceous earth. The clear filtrate was passed through a Berkefeld filter to remove all the bacteria present. The technique used was similar to that recommended by d'Hérelle.<sup>3</sup> The organism was isolated and in addition stock strains of *Bacillus carotovorus*, *B. melonis*, and *B. coli* were now inoculated into tubes of plain broth and incubated for one hour. Varying amounts of the

<sup>1</sup> Received for publication Mar. 10, 1924.

<sup>2</sup> This work was done in the spring of 1922. Since writing this paper, the following article has appeared: GERRETSEN, F. C., AND OTHERS. DAS VORKOMMEN EINES BAKTERIOPHAGEN IN DEN WURZELKNÖLLCHEN DER LEGUMINOSEN. Centbl. Bakt., (II) 60: 311-316, illus. 1923.

<sup>3</sup> D' HÉRELLE, F., THE BACTERIOPHAGE, ITS RÔLE IN IMMUNITY. . . . Tr. by G. H. Smith. 287 p., illus. Baltimore. 1922.

sterile filtrate were then added to these tubes, as shown in Table I. The results were obtained in 48 hours. The tubes of the cabbage-rot organisms were now filtered as before and the filtrate again added to fresh broth cultures, with the results shown in Table II.

As Tables I, II, and III indicate, the second transfer shows a marked improvement in the inhibition of the organism and the third transfer shows an even more decided improvement over the second. In other words, a marked development of the inhibitory substance resulted from invigoration by transplanting.

TABLE II.—*The effect of the cabbage-rot organism after one invigoration*

Culture	Control	Amount of filtrate added		
		1 drop	2 drops	2 cc.
<i>B. carotovorus</i> .....	—	—	—	—
Do.....	—	±	±	±
Cabbage-rot organism.....	—	+	+	+
Do.....	—	+	+	+

<sup>a</sup> The following symbols are used in the tables: —, very strong turbidity; ±, slightly less turbidity; +, cloudy; ++, less cloudy; +++, nearly clear; +++++, clear.

In the same manner a third set was prepared with the following results (Table III):

TABLE III.—*The effect of the cabbage-rot filtrate after two invigorations*<sup>a</sup>

Culture	Control	Amount of filtrate added		
		1 drop	2 drops	2 cc.
Cabbage-rot organism.....	—	+++++	++	+++++
Do.....	—	+++++	++	+++++

<sup>a</sup> The following symbols are used in the tables: —, very strong turbidity; ±, slightly less turbidity; +, cloudy; ++, less cloudy; +++, nearly clear; +++++, clear.

This substance is called an inhibitory substance because the tubes containing the filtrate remained clear for 48 hours and then slowly showed growth. The control tubes, on the other hand, were turbid in 24 hours. In no case was any evidence of lysis apparent, all the tubes showing only a retardation of growth for at least 48 hours.

The type of growth appearing in the tubes containing the filtrate was very interesting. The tubes after 48 hours would show a thin pellicle on the surface with a clear liquid beneath and no sediment. Upon agitation, this pellicle would break into granules, which would sink to the bottom of the tube. Upon shaking the tubes, the granules would break up and a persistent turbidity would result. The control tubes, on the other hand, would show a persistent turbidity in 24 hours without pellicle or sediment. The filtrate apparently induced this change of growth to take place.

Unpublished work by one of the authors (Mallmann) showed exactly the same occurrence in broth with *Bacillus coli* where an inhibitory substance of like nature was added. Plates from tubes of cabbage-rot organism showed only the typical normal colonies. These differ from the plates of *B. coli* where two types were found, a normal colony and a so-called "rough" colony, as shown by Bergstrand.<sup>4</sup>

After repeated transfers, the inhibitory substance adapted itself to some extent to other related organisms. It inhibited *Bacillus spieckermani* and *B. carotovorus*

<sup>4</sup> Bergstrand, H. ON THE VARIATIONS OF BACTERIUM COLI. *Jour. Bact.* 8: 173-192, illus. 1923.

TABLE IV.—The effect of the cabbage-rot filtrate after repeated invigorations<sup>a</sup>

Culture	Control	Amount of filtrate added		
		1 drop	2 drops	2 cc.
Cabbage-rot organism.....	—	++++	++++	++++
Do.....	—	++++	++++	++++
<i>Bacillus speckermani</i> .....	—	—	—	++++
Do.....	—	—	—	++++
<i>Bacillus carotovorus</i> .....	—	—	—	++++
Do.....	—	—	—	++++
Potato-rot bacillus.....	—	—	—	—
Do.....	—	—	—	—

<sup>a</sup> The following symbols are used in the tables: —, very strong turbidity; ±, slightly less turbidity; +, cloudy; ++, less cloudy; +++, nearly clear; +++++, clear.

TABLE V.—The effect of heating the filtrate at 56° C. for 30 minutes<sup>a</sup>

Culture	Control	Amount of filtrate added		
		1 drop	2 drops	2 cc.
Cabbage-rot organism.....	—	+	++	++++
Do.....	—	±	±	±

<sup>a</sup> The following symbols are used in the tables: —, very strong turbidity; ±, slightly less turbidity; +, cloudy; ++, less cloudy; +++, nearly clear; +++++, clear.

TABLE VI.—The effect of heating the filtrate at 63° C. for 30 minutes<sup>a</sup>

Culture	Control	Amount of filtrate added		
		1 drop	2 drops	2 cc.
Cabbage-rot organism, unheated filtrate.....	—	++++	++++	++++
Do.....	—	++++	++++	++++
Cabbage-rot, heated filtrate.....	—	—	—	—
Do.....	—	—	—	—

<sup>a</sup> The following symbols are used in the tables: —, very strong turbidity; ±, slightly less turbidity; +, cloudy; ++, less cloudy; +++, nearly clear; +++++, clear.

where a large amount of filtrate was added, but refused to inhibit the potato-rot bacillus. A filtrate made two weeks later from these tubes failed to inhibit *B. speckermani* and *B. carotovorus* but was still very active against the cabbage-rot bacillus.

## RESISTANCE OF THE INHIBITORY SUBSTANCE TO HEAT

An active filtrate was heated to 56° C. for 20 minutes and then added as usual to the cabbage-rot bacillus.

The heating at 56° C. for 20 minutes decreases the activity of the inhibitory substance but it was still active enough to show partial inhibition in one case.

A second active filtrate was now heated to 63° C. for 30 minutes and added as usual to young organisms in broth.

This temperature caused a complete destruction of the inhibitory substance as shown in the above table. This checks very favorably with previous work by others working on similar substances isolated from animal sources.



## CONCENTRATION OF THE INHIBITORY SUBSTANCE

An active filtrate was added to broth cultures of young cabbage-rot bacillus in dilutions starting with 1 to 10 and progressing by dilutions of 10 up through 12 dilutions, or to a final dilution of 1 to 1,000,000,000,000. The tubes of broth contained 9 cc. of broth. To the first tube was added 1 cc. of filtrate making a 1-to-10 dilution. Using the same pipette, 1 cc. was taken from this tube to the second, and so on through the set. All of these tubes showed inhibition through a dilution of 1 to 100,000,000,000.

After a period of several months, the above experiment was repeated, using higher dilutions. The dilutions this time were run up by dilutions of 10 to a final dilution of 1 to 1,000,000,000,000,000,000. The filtrate still showed inhibition at a dilution of 1 to 100,000,000,000,000,000. The last dilution failed to show any inhibition.

The dilution in which the inhibitory substance is active is extremely high, far higher than could be induced through any toxic material produced by the organism. There is but little doubt that the material isolated is comparable to inhibitory substances obtained from animal sources.

The organism upon which the inhibitory substance was active proved upon identification to belong to the fluorescent group rather than *Bacillus carotovorus*.<sup>5</sup> However, this organism does decompose cabbage. Experiments to determine its decomposing power showed a slow rotting, several weeks being required for complete liquefaction of the cabbage.

Filtrates made from the pure culture never showed the inhibitory substance nor did the culture of *Bacillus carotovorus*. This proves that the inhibitory substance must have come from the cabbage. Filtrates from normal cabbage failed to show the inhibitory substance, which indicates an association of the inhibiting substance with the disease-producing organism. This is similar to conditions in the intestinal tract of animals as demonstrated by d'Hérelle.<sup>6</sup>

## CONCLUSIONS

An inhibitory substance was isolated from a rotten cabbage which was active on an organism obtained from the same cabbage.

The inhibitory substance became active against other soft rot-producing organisms but this activity was lost by further transplanting.

The inhibitory substance was not destroyed at 56° C. for 20 minutes, but was destroyed at 63° for 30 minutes, showing a sensitiveness to heat comparable to microorganisms and lytic substances isolated from animal sources.

The inhibitory substance was present in extremely large amounts, as indicated by its activity in high dilution. It was therefore probably not a toxic product of the organism.

Lytic and inhibitory substances are probably found in plants as in animals. It is hoped that this brief preliminary report will open up this field and show the extent of lytic and inhibitory substances in the plant world.

<sup>5</sup> The organism is approximately the same size and shape as *Bacillus carotovorus*. It occurs singly and is sluggishly motile; Gram-negative, no gas produced in dextrose, lactose, or saccharose broth. Plain broth becomes decidedly turbid in 24 hours with a slight pellicle appearing in six to seven days, at which time a yellowish green coloration appears near the surface. The agar slant shows an abundant white opaque growth with no discoloration of the medium. Gelatin is liquefied.

<sup>6</sup> D'HÉRELLE, F., THE BACTERIOPHAGE, ITS RÔLE IN IMMUNITY. . . . tr. by G. H. Smith. 287 p., illus. Baltimore. 1922.

## TWO HITHERTO UNREPORTED DISEASES OF STONE FRUITS <sup>1</sup>

By C. C. LINDEGREN, *Field Assistant*, and DEAN H. ROSE, *Pathologist, Fruit Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*

### BOTRYTIS ROT OF PEACHES

In August, 1923, during the inspection of peach shipments from California, on the Chicago market, numerous fruits were found which showed a rot that was apparently due to *Botrytis*. On nearly all of these fruits there was a scanty growth of white to gray mycelium, and on a few of them the characteristic spore clumps of *Botrytis* could be seen. The varieties found attacked were Elberta and Lovell.

Cultures made under aseptic conditions from the advancing edge of the rotten spots yielded a *Botrytis* which was apparently very similar to *B. cinerea*. Positive evidence of the pathogenicity of the organism isolated was obtained in an experiment carried out as follows: Ripe healthy peaches were immersed for two minutes in 50 per cent alcohol, then rinsed thoroughly with sterilized distilled water; four of the peaches were inoculated by wounding with a flamed needle and forcing mycelium into the wound, four others were inoculated by merely laying a fragment of mycelium on the uninjured peach surface. For controls, four peaches were used which had received no treatment other than sterilization, and four others which after sterilization had been merely wounded with a flamed needle. All four of these lots were placed in moist chambers and held at room temperature. Within three days all of the peaches in the two inoculated lots showed light brown rotten spots about 2 cm. in diameter; by the end of eight days the spots had reached a diameter of 10 cm. Cultures from these spots yielded a *Botrytis* which was apparently identical with the one used for inoculations.

Similar results from inoculations were obtained in several other experiments conducted in the same way. The controls in all of the experiments failed to develop rot of any sort.

The noteworthy fact which these experiments establish is that *Botrytis* is able to penetrate the uninjured skin of the peach and that when it does so it can produce rot as rapidly as if the fruit had been wounded.

In lesions caused by *Botrytis*, peach tissue, both skin and flesh, is firmer in texture and lighter brown in color than in lesions caused by either *Sclerotinia* or *Rhizopus*. The skin around the margin of the lesion slips easily under pressure from the finger somewhat as in *Rhizopus* rot, but the decayed tissue does not have the marked sour odor characteristic of *Rhizopus* rot. As a matter of fact it has no definite odor of any sort. So far as noted there is no tendency to wrinkle and mummify.

### ALTERNARIA ROT OF CHERRIES

During the summer and fall of 1923 the most conspicuous, although probably not the most destructive, disease of cherries on the Chicago market was a brown decay with abundant olive green, sporulating, aerial mycelium. Bing sweet cherries from Yakima and Wenatchee, Washington, and Lambert sweet cherries from Emmett, Idaho, showed a large amount of this rot. It was also found on

<sup>1</sup> Received for publication Apr. 1, 1924.

Windsor sweet cherries from northern Michigan and on Montmorency (sour) and Early Richmond (sour) cherries from southern Michigan. The symptoms on sour and sweet cherries are decidedly dissimilar.

On the sweet cherry the rot is characterized by a firm, brown, cone-shaped mass extending in toward the pit. This firm, brown mass is made up of dead host cells interpenetrated and apparently held together by the fungous mycelium. It is firmer than the surrounding tissue, and if the skin that covers it be carefully broken the affected tissue beneath can often be lifted out intact. The skin which overlies the affected area is covered with an olive-green growth of sporulating mycelium; sometimes, however, this sporulating layer is hidden under a mass of white, fluffy mycelium.

The symptoms on sour cherry are quite different. The only similarity, in fact is the olive-green layer of sporulating mycelium growing on the epidermis, and even this soon becomes soaked with juice and looks black and matted. In the early stages of the disease there is apparently so little penetration of the mycelium below the epidermis that the whole surface of a cherry may be covered with the olive-green growth before the flesh is decayed near the pit. When the tissue decay does take place, it is light brown and not nearly so firm as that in the sweet cherry. No fluffy growth of mycelium has been observed on sour cherries by the writers. In general, the disease on sour cherries is characterized by a decayed epidermis covered with a black, water-soaked mat of sporulating mycelium. The stem of the decayed cherry is almost invariably dead and often contains fungi apparently saprophytic, notably *Cladosporium* sp. and *Botrytis* sp.

The olive-green decay is caused by *Alternaria* sp., which apparently enters through wounds in the cherry. These wounds during 1923 consisted mainly of cracks, the cause of which is entirely problematical. It is possible they were rain cracks, though certain circumstances suggest that they may arise in transit. The question is one which needs further investigation. Numerous isolations were made from collected specimens and several different organisms were obtained, namely, unidentified species of *Rhizopus*, *Penicillium*, *Botrytis*, *Mucor*, *Aspergillus*, and *Cladosporium*. Most of these organisms proved pathogenic, but lesions like those described above, particularly those described for sweet cherries, were produced only by inoculations with the *Alternaria* obtained from diseased cherries. There is no definite proof that the species of *Alternaria* isolated from the two kinds of cherries are identical. Cross-inoculations have shown, however, that the *Alternaria* from sweet cherries is pathogenic to sour cherries, and vice versa.

Inoculation experiments were set up as follows: The stems, which were usually dead and contained various species of fungi, were clipped and the cherries were sterilized in a 1 to 1,000 solution of mercury bichlorid, or in 50 per cent alcohol, for 5 to 30 minutes. They were then rinsed in sterilized tap water and placed in large sterile Petri dishes. Inoculations were made either (1) by forcing mycelium from an agar culture of *Alternaria* into the flesh of the cherries with a sterile needle, or (2) by merely laying fragments of mycelium on the surface of uninjured cherries. Infection was obtained in each of several hundred inoculations made by the first method and always resulted in lesions like those described above; from these lesions, on both sweet and sour cherries, an *Alternaria* was reisolated which was apparently identical with the one used for inoculation. No infection resulted from inoculations made by the second method. Failure to obtain it is taken to mean that the fungus is unable to penetrate the uninjured skin of the fruit. No infection developed on the controls, which consisted of wounded and unwounded cherries previously sterilized in the manner already described. All experiments were conducted at room temperature.

## SUMMARY

There has been found, on peaches shipped from California, a rot from which a fungus similar to *Botrytis cinerea* has been isolated. It is proved, by inoculation and reisolation, that this fungus is the cause of the disease. The inoculation experiments indicate that the fungus is able to penetrate the uninjured skin of the fruit.

There has been found on sweet cherries shipped from Michigan a rot from which a species of *Alternaria* has been isolated. There is no proof that the *Alternaria* from sour cherries is identical with that from sweet cherries, but cross-inoculation and reisolation have shown that both strains are pathogenic to both kinds of cherries. The inoculation experiments indicate that the fungus is unable to penetrate the uninjured skin of the fruit.

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# JOURNAL OF AGRICULTURAL RESEARCH

## CONTENTS

Studies Relating to the Immunology of Bovine Infectious Abortion - - - -	Page 607
J. M. BUCK and G. T. CREECH	
The Rhizoctonia Brown Rot and Other Fruit Rots of Strawberries - - - -	643
B. O. DODGE and NEIL E. STEVENS	
Effective Use of Hydrocyanic-Acid Gas in the Protection of Chick-Peas ( <i>Cicer arietinum</i> ) Warehoused in 240-Pound Sacks - - - -	649
E. A. BACK and R. T. COTTON	
A New Nematode, <i>Cylindropharynx ornata</i> , from the Zebra, with Keys to Related Nematode Parasites of the Equidae - - - -	661
ELOISE B. CRAM	
Chromosomes in Maize and Maize Relatives - - - -	673
ALBERT E. LONGLEY	
A Fusarium Bulb Rot of Onion and the Relation of Environment to Its Development -	683
J. C. WALKER and E. C. TIMS	
The Chlorid Content of the Leaf Tissue Fluids of Egyptian and Upland Cotton - -	695
J. ARTHUR HARRIS, JOHN V. LAWRENCE, and ZONJA WALLEN LAWRENCE	
The Resistance of Oat Varieties to Stem Rust - - - -	705
WILLIAM W. MACKIE and RUTH F. ALLEN	

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# JOURNAL OF AGRICULTURAL RESEARCH

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No. 7

## STUDIES RELATING TO THE IMMUNOLOGY OF BOVINE INFECTIOUS ABORTION<sup>1</sup>

By J. M. BUCK and G. T. CREECH, *Pathological Division, Bureau of Animal Industry, United States Department of Agriculture*

### INTRODUCTION

Artificial immunization as a means of combating infectious diseases of the domestic animals is a subject that during recent years has received no little amount of consideration. Numerous diseases which formerly exacted heavy losses are now being more or less successfully suppressed through the utilization of immunizing substances. As a result of the progress made along these lines, persons interested in disease-control work have naturally been much encouraged and led to entertain hope that such methods of combating disease may in the course of time be greatly extended.

### REVIEW OF PREVIOUS WORK

Investigators for a number of years have been interested in determining whether bovine infectious abortion may be advantageously attacked through the agency of biological preparations. The subject has been of especial interest in connection with this disease because of the fact that the control of *Bacterium abortus* exposure in infected herds is extremely difficult and because the total elimination of the infection from such herds frequently is accomplished only at a financial sacrifice which renders such an attempt impracticable.

Efforts to evolve and evaluate biological methods of control have been prompted to a great degree by the observation that in recently infected herds the disease generally spreads rapidly and for a period causes excessive losses which, in the event that animals from other herds are excluded, show a tendency to gradually subside, or in the course of time even largely to disappear. It has seemed reasonable to ascribe the improvement in conditions, in part at least, to the development of a naturally acquired immunity and to infer that a resistance of similar character should be possible of development in an artificial manner.

Bang<sup>2</sup> in his early writings relative to the isolation of the abortion microorganism called attention to the possibilities of artificial immunization, and in a later report<sup>3</sup> recorded the results of his experiments in which both living and killed cultures of *Bacterium abortus* were employed. A practice was made of injecting nonpregnant animals either subcutaneously or intravenously a number of times and, following conception, subjecting them to a severe type of *Bacterium abortus* exposure. While many of his treated animals failed to withstand the degree of exposure to which they were subjected, the results obtained were nevertheless encouraging, particularly those derived from the subcutaneous administrations of living cultures.

<sup>1</sup> Received for publication Mar. 4, 1924.

<sup>2</sup> BANG, B.—DIE AETIOLOGIE DES SEUCHENHAFTEN ("INFECTIÖSEN") VERWERFENS. Ztschr. Tiermed. 1: 241-278, illus. 1897.

<sup>3</sup> BANG, B.—INFECTIOUS ABORTION IN CATTLE. Jour. Compar. Path. and Ther. 19: 191-202. 1906.



McFadyean and Stockman<sup>4</sup> in their early investigations of the disease also gave the matter of artificial immunization attention and described in detail experimental work dealing with two virgin heifers that received subcutaneously single massive doses of *Bacterium abortus* cultures. These animals were later bred and during their gestation periods received enormous amounts of material containing abortion bacteria. When destroyed in an advanced stage of pregnancy failure was experienced in detecting pathological changes in their uteri or in isolating therefrom the abortion organism. Such findings led these workers to conclude that the treatment practiced preceding conception conferred an active immunity.

Stockman<sup>5</sup> in discussing infection abortion at a later date gave further consideration to artificial immunization as a means of preventing abortion losses. He described results that were obtained in infected herds where single massive doses of living cultures or suspensions of *Bacterium abortus* were administered to nonpregnant animals and where heavy suspensions of killed abortion bacteria were administered to the pregnant animals at monthly intervals up to the sixth month of pregnancy. Instead of making use of chemical agents for killing the organisms, as was practiced by Bang in his preparation of bacterins, the suspensions were subjected to a temperature of 65° C. for one-half hour. Those animals receiving the viable organisms were not bred for a period of at least two months following the injections. Badly infected herds are mentioned as having been selected for experimentation. An estimate of the value of the immunizing procedure was arrived at by comparing the number of abortions that subsequently occurred in the treated groups with those that occurred in control animals in the same herds. With this method of evaluation the results derived from the employment of living abortion bacteria appeared to be highly encouraging, for whereas 23.4 per cent of the 432 controls aborted, the abortion rate in the 493 treated subjects was but 6.5 per cent. Inasmuch as 21 per cent of 110 animals that received repeated injections of killed organisms aborted, it was concluded that the benefits derived from the use of the killed organisms was not sufficiently marked to compensate for the labor involved in the making of the numerous injections.

Investigators in this and foreign countries, other than those previously mentioned, who have contributed data bearing on the subject of artificial immunization include the following: Giltner, Hallman, Williams, Hadley, Huddleson, Barnes, Hagan, Klimmer, Zwick and his coworkers, and others.

The results of immunizing experiments that have been reported from time to time have for the most part been disappointing where either killed abortion bacteria or serum with a high agglutinating titer for abortion bacteria have been utilized as immunizing agents. On the other hand, when viable abortion bacteria have been employed in connection with nonpregnant subjects, the resistance of the animals to the pathogenic action of *Bacterium abortus* has subsequently appeared to be materially increased.

### THE PRESENT EXPERIMENTS

The experimental work recorded in this paper, which was inaugurated during the latter part of 1917, was prompted largely by the encouraging results previously mentioned as having been reported by Stockman in connection with the extensive immunizing work carried out in England. While the principal object

<sup>4</sup> MCFADYEAN, J., and STOCKMAN, S.—REPORT OF THE DEPARTMENTAL COMMITTEE APPOINTED BY THE BOARD OF AGRICULTURE AND FISHERIES TO INQUIRE INTO EPIZOOTIC ABORTION. Appendix to Part I. 43 p. London. 1909.

<sup>5</sup> STOCKMAN, S.—EPIZOOTIC ABORTION. Internat. Vet. Cong. Rpt. 10 (2). 343-354. 1915.

in view in undertaking the work was that of procuring additional data with reference to the possibilities of immunization, it was also planned to give some consideration to the length of time that animals treated with living abortion organisms carry the infection in their bodies as a result of subcutaneous inoculations. That many noninfected animals might thereby be made carriers or disseminators of the disease seemed possible, in view of the fact that a rather high percentage of cows which acquire the disease under natural conditions have been shown to harbor the infection in their udders for long periods, and frequently eliminate the bacteria from their genital tracts at subsequent parturitions irrespective of whether they expel dead or seemingly normal calves. It was proposed in the immunizing experiments to employ both living and killed *Bacterium abortus* organisms, although the value of the latter in rendering animals resistant to infection had been seriously questioned by different investigators.

#### METHODS OF PROCEDURE

Two general methods of procedure were followed in applying the immunizing tests. In one of these use was made of infected herds where a portion of the animals were subjected to immunizing treatments and the remainder employed as controls. An attractive feature of this method was that it made available for experimentation a large number of animals. Bacteriological studies of aborting cows could, however, seldom be made, in consequence of which the interpretation of results was rendered somewhat complex and confusing. Impossibility of controlling *Bacterium abortus* exposure of individual animals under herd conditions, moreover, introduced another element of uncertainty.

In the second method of procedure the number of animals used was limited, but it was possible to give more attention to their selection and handling than could be done under herd conditions where experimental results were secondary. Care was exercised in the procuring of stock which was susceptible to the disease. Serological tests were conducted at frequent intervals, and cultural and guinea-pig inoculations of milk and uterine materials were made at such times as were considered advantageous or instructive. The results that were thereby obtained are regarded as being of greater interest and significance than those derived from the experiments carried out under herd conditions, and will therefore be discussed somewhat more in detail.

#### EXPERIMENTS WITH SELECTED STOCK

The stock referred to, consisting of 66 females and 15 bulls ranging from 1 to 3 years of age, was obtained from an isolated mountainous section of Tennessee into which, so far as could be determined, infectious abortion had not been introduced. Approximately 20 per cent of the heifers had been bred when purchased. Upon subjecting the entire shipment to the complement-fixation and agglutination tests negative reactions were obtained in all cases; that is, no fixation of complement and no agglutination of a *Bacterium abortus* suspension was produced by 0.04 cc. or lesser amounts of blood serum from any animal. Freedom from *Bacterium abortus* infection was further evidenced by the fact that no symptoms of abortion disease were manifested by any of the pregnant heifers that were permitted to complete their gestation periods before being used for experimental purposes.

Of the shipment described, 18 heifers and 5 cows were used in the experiment. Eight heifers and 3 cows received subcutaneous administrations of abortion vaccine<sup>6</sup> when nonpregnant, 4 heifers received abortion bacterin, and 6 heifers and 2 cows were used as controls.

<sup>6</sup> The term "vaccine" as used in this paper has reference to a suspension of living *Bacterium abortus*, while "bacterin" is used to indicate killed suspensions of the organism.

A 20-cc. dose has been used in practically all immunizing work that has been undertaken with living abortion bacteria, whether under herd conditions, where natural *Bacterium abortus* exposure has prevailed, or in connection with animals that have been utilized in a purely experimental capacity. This quantity of vaccine represents one-eighth of the growth of abortion organisms developing on approximately 15 square inches of 3 per cent glycerin-agar after seven days' incubation at a temperature of 37.5° C. When dilutions of the vaccine have been plated the number of colonies that have developed has indicated that the product contained approximately one billion organisms per cubic centimeter. Fifteen to twenty Blake bottles of medium have usually been sown at one time with suspensions of as many different strains, and a practice has been made of using only such strains as have been artificially cultivated for at least a year. After seven days' incubation the growth in each bottle was suspended in 160 cc. physiological salt solution. While there has been some variation in the amount of growth that has developed in the different bottles, vaccine of fairly uniform density has been obtained by combining the suspensions of each lot. The quantity of vaccine administered to each animal has been considerably less than that mentioned by Stockman, who refers to the organisms obtained from a similar area of medium as constituting a dose for but one or two subjects.

The vaccinated animals were mated with a bull that was used exclusively for serving those so treated and which gave negative complement-fixation and agglutination reactions throughout the experiment. The bull that was mated with the bacterin-treated group, as well as those to which the controls were bred, were protected from abortion exposure, and gave negative reactions to the serological tests at all times.

The animals receiving bacterin were treated as follows: Two heifers, No. 423 and 404, received subcutaneous injections two and four times, respectively, before being bred, and five times each before being subjected to *Bacterium abortus* exposure. The injections were continued monthly in doses of 20 cc. The suspensions were identical in density with the vaccine described above, but were placed in a water bath and maintained at a temperature of 65° C. for one-half hour. One heifer, No. 464, was treated bimonthly with half the dose of killed organisms and received two injections previously and seven subsequently to exposure. The fourth heifer, No. 401, received bimonthly injections of 20 cc., two of which were given previously to exposure but subsequently to breeding date. This animal received seven treatments in all. Individual stalls were provided for the treated as well as the control animals during their gestation periods, and all the animals were handled in such a manner as to reduce to a minimum the possibility of contact with *Bacterium abortus* infection other than that intentionally furnished.

The method of exposure to *Bacterium abortus* was as follows: Varying quantities, from 5 to 60 cc., of stomach contents of aborted fetuses, from which *Bacterium abortus* and no other microorganisms could be isolated, were administered by the mouth. The material was diluted with from 50 to 100 cc. of physiological salt solution. One feeding was practiced for the most part, although in a few instances it was considered desirable to administer the material a second time. While numerous lots of stomach contents were required for the exposure of the 23 animals utilized, by reason of the fact that the breeding dates extended over a period of considerable length, efforts were made to breed the animals at such times as would permit of the exposure of those treated and of the controls being as nearly uniform in character as possible.

## RESISTANCE OF TREATED AND CONTROL ANIMALS

During the first gestation period 10 of the 11 vaccinated animals produced living calves. One abortion occurred in this group, heifer No. 447, from the fetus of which *Bacterium abortus* was isolated. Another animal, cow No. 410, produced a living calf at the expiration of a 229-day gestation period. When she was slaughtered ten days later *Bacterium abortus* was isolated from her uterus. Failure was experienced in demonstrating the presence of *Bacterium abortus* in the placentas or uterine fluids of any of the other 9 animals of this group at time of parturition, although cultural efforts and guinea-pig inoculations were practiced in all cases.

In the bacterin-treated group 2 heifers, No. 423 and 401, produced living calves at the expiration of gestation periods of 282 and 273 days, respectively. Failure was experienced in isolating *Bacterium abortus* from the placentas or uterine fluids of these 2 animals at time of parturition. Bacterin-treated heifers 404 and 464 expelled dead calves that were shown to harbor *Bacterium abortus* infection.

Any comparison of the amount of exposure in these cases with that sustained by animals in infected herds under natural conditions is more or less conjectural, but that it was severe is evidenced by the fact that 7 of the 8 controls subsequently aborted, and *Bacterium abortus* was isolated from the fetuses, placentas, or uterine fluids of all 8 subjects.

Table I shows the dates of vaccination, the intervals between treatment and breeding dates, the time that elapsed between conception and exposure, the length of the gestation periods, and the final results obtained.

TABLE I.—Immunization results in animals subjected to artificial exposure to *Bacterium abortus*

## TREATED WITH ABORTION VACCINE

Experimental animal	Date of treatment	Date of breeding	Date of exposure	Exposure material (fetal-stomach contents)		Gestation period	Outcome of pregnancy	Results of uterine examination for <i>Bact. abortus</i>
				Case No.	Amount			
Heifer 402.....	Mar. 2, 1917	Apr. 27, 1917	Nov. 2, 1917	42	5	276	Living calf..	Negative.
		Aug. 29, 1918	Nov. 13, 1918	74	50	279	do.....	Do.
		Oct. 3, 1919	Mar. 18, 1920	110	20	280	do.....	Do.
Heifer 409.....	Mar. 2, 1917 Mar. 23, 1917	Apr. 3, 1917	June 8, 1917	21	50	282	do.....	Do.
		July 24, 1918	Aug. 20, 1918	70	25	280	do.....	Do.
		Oct. 24, 1919	Mar. 18, 1920	110	20	284	do.....	Do.
Heifer 417.....	Feb. 1, 1917	May 25, 1917	Dec. 13, 1917	54	30	272	do.....	Do.
		July 12, 1918	Aug. 20, 1918	70	30	267	do.....	Do.
		Oct. 24, 1919	Mar. 18, 1920	110	20	279	do.....	Do.
Heifer 418.....	Mar. 2, 1917 Mar. 23, 1917	July 25, 1918	Aug. 20, 1918	70	25	284	do.....	Do.
		Oct. 8, 1919	Mar. 18, 1920	110	20	277	do.....	Do.
			May 10, 1920	126	22		do.....	Do.
Heifer 419.....	Mar. 30, 1918	Dec. 30, 1918	Feb. 24, 1919	80	60	281	do.....	Do.
			Mar. 19, 1919	88	25	281	do.....	Do.
			May 10, 1920	126	22	282	do.....	Do.
Heifer 427.....	Mar. 2, 1917	Apr. 21, 1920	June 8, 1917	21	50	282	do.....	Do.
		Apr. 6, 1917	June 8, 1917	21	50	282	do.....	Do.
		July 17, 1918	Aug. 20, 1918	70	25	278	do.....	Do.
Heifer 462.....	Jan. 10, 1919	Oct. 3, 1919	Mar. 18, 1920	110	20	282	do.....	Do.
		Mar. 12, 1919	May 10, 1920	126	22	285	do.....	Do.
		Apr. 26, 1920	May 10, 1920	126	22	284	do.....	Do.
Heifer 447.....	Mar. 30, 1918	July 1, 1918	Nov. 13, 1918	74	30	259	Abortion..	Positive.
Cow 408.....	Feb. 24, 1919	Apr. 12, 1919	May 24, 1919	92	50	283	Living calf..	Negative.
Cow 410.....	Mar. 2, 1920	Apr. 22, 1920	May 10, 1920	126	22	229	Weak calf..	Positive.
Cow 416.....	Mar. 2, 1920	May 5, 1920	May 10, 1920	126	22	290	Living calf..	Negative.

TABLE I.—Immunization results in animals subjected to artificial exposure to *Bacterium abortus*—Continued

## TREATED WITH ABORTION BACTERIN

Experimental animal	Date of treatment	Date of breeding	Date of exposure	Exposure material (fetal stomach contents)		Gestation period	Outcome of pregnancy	Results of uterine examination for <i>Bact. abortus</i>
				Case No.	Amount			
Heifer 423.....	Feb. 1, 1917 Mar. 2, 1917 Apr. 2, 1917 May 2, 1917 June 7, 1917 July 2, 1917 Aug. 4, 1917 Sept. 4, 1917 Oct. 5, 1917 Nov. 3, 1917 Dec. 3, 1917	Mar. 23, 1917	June 8, 1917	21	Cc. 50	Days 282	Living calf..	Negative.
Heifer 401.....	Dec. 7, 1917 Jan. 2, 1918 Jan. 22, 1918 Feb. 2, 1918 Mar. 4, 1918 Mar. 25, 1918 Apr. 15, 1918	June 5, 1918 Aug. 8, 1917	Aug. 20, 1918 Jan. 5, 1918	70 54	25 30	231 273	Abortion.... Living calf..	Do. Do.
Heifer 404.....	Feb. 1, 1917 Mar. 1, 1917 Apr. 2, 1917 May 2, 1917 June 2, 1917 July 2, 1917 Aug. 4, 1917 Sept. 4, 1917 Oct. 5, 1917	Sept. 23, 1918 Oct. 14, 1918 May 24, 1917	Nov. 3, 1918 June 7, 1917	74 21	20 50	278 162	-----do----- Abortion....	Do. Positive.
Heifer 464.....	Oct. 5, 1917 Oct. 20, 1917 Nov. 2, 1917 Nov. 16, 1917 Dec. 3, 1917 Dec. 18, 1917 Jan. 2, 1918 Jan. 31, 1918 Feb. 14, 1918	June 25, 1917	Nov. 2, 1917	42	5	238	-----do-----	Do.

## CONTROLS

Heifer 446.....	May 4, 1917	June 8, 1917	21	50	182	Abortion....	Positive.
Heifer 422.....	May 4, 1917	June 8, 1917	21	50	177	-----do-----	Do.
Heifer 460.....	June 16, 1917	Nov. 2, 1917	42	5	265	-----do-----	Do.
Heifer 450.....	Aug. 16, 1917	Dec. 13, 1917	54	30	241	-----do-----	Do.
Heifer 413.....	May 9, 1917	June 8, 1917	21	50	206	-----do-----	Do.
Heifer 430.....	Jan. 7, 1919	{Feb. 24, 1919 Mar. 19, 1919	80 88	20 25	223	-----do-----	Do.
Cow 470.....	Feb. 24, 1919	May 24, 1919	92	50		Living calf..	Do.
Cow 453.....	Dec. 18, 1919	May 10, 1920	126	22	186	Abortion....	Do.

The source of the fetal-stomach contents used for exposure purposes is indicated in Table I in order that animals receiving similar exposure may be readily identified. The fetal material was transferred to sterile flasks at the times the fetuses were cultured and was held at refrigerator temperature until cultural results were ascertained.

It may be observed by reference to Table I that seven vaccinated animals were carried through a second period of pregnancy without further administration of vaccine and that living calves were produced in all cases. Failure was furthermore experienced in demonstrating the presence of *Bacterium abortus* in the placentas or uterine fluids of these animals at time of parturition. Four of the

animals were carried through a third period and gave further evidence of immunity, after a third exposure, by producing living calves and by the absence of uterine infection as indicated by the cultural and guinea-pig inoculation work that was carried out.

It may also be observed by reference to Table I that but 3 cows were subjected to the vaccination procedure, the remaining 8 being heifers. The udders of these 3 were shown to harbor *Bacterium abortus* at time of parturition. The presence of the infection in 2 of these subjects, 408 and 416, was not demonstrated previously to the ingestion exposure, in consequence of which it can not be assumed that the vaccine treatment was responsible for the condition. *Bacterium abortus* was, however, isolated from the milk of cow No. 410 forty-four days subsequently to vaccination and previously to date of ingestion exposure. Although this animal produced a living calf at the expiration of a 229-day gestation period, the vaccine treatment is looked upon as probably having been responsible for both the premature expulsion of the fetus and the presence of *Bacterium abortus*, which was shown to exist in her uterus at time of parturition.

Animals No. 423 and 401, which gave evidence of having derived some protection from treatment with bacterin, were carried through a second pregnant period without further efforts at immunization. Animal No. 423 had received 11 and animal No. 401, 7 injections of killed abortion bacteria previously to first pregnancy. Cow No. 423 aborted at the expiration of 231 days, but failure was experienced in demonstrating the presence of *Bacterium abortus* in either the fetus or the other products of the abortion. *Bacterium abortus* infection of the udder was demonstrated in cow No. 401 during and at termination of this pregnancy, although its presence in the uterus at time of parturition could not be established.

#### OBSERVATIONS WITH REGARD TO SEROLOGICAL TESTS

Bimonthly applications of the serological tests to the blood serum of the animals used in the experiment proved to be of interest. It could reasonably be inferred from the character of the reactions obtained that in none of the vaccinated heifers did *Bacterium abortus* infection persist for any great length of time following treatment. In these animals it was observed that a decrease in agglutinins and complement-fixing bodies began soon after vaccination and gradually continued until their blood serum exhibited slight or no agglutinating or complement-fixing properties. Cow No. 410 was the only vaccinated animal that continued to react in a pronounced manner to the tests during the entire gestation period, and, as previously stated, she harbored the infection in her udder 44 days subsequently to the treatment.

The charts of the individual animals (Table V, p. 621) show some degree of variation in the results of the agglutination tests that were obtained at the different testing dates, as well as between the agglutination and complement-fixation reactions, particularly in those animals that received injections of abortion vaccine. The blood serum of these animals usually possessed some agglutinating properties in the quantities employed, when complement-fixing bodies could no longer be demonstrated. The results obtained made it appear evident that slight reactions to the agglutination test may persist for long periods after infection of the animal has apparently been overcome. It is somewhat questionable, however, whether the agglutinin content of the sera from the treated animals varied from time to time to the extent indicated by the results recorded. Slight variations in density of the test fluid employed, a difference in the age of the suspensions, a certain degree of variation in the length of the period that the tests were incubated, and the personal factor involved in the reading of the tests were features that singly or in combination may have been largely responsible for such variations in results as the charts indicate.

The quantities of serum that were used in making the agglutination tests were 0.04, 0.02, 0.01, 0.005, 0.002, and 0.001 cc. To these quantities were added 1 cc. of a suspension of abortion organisms similar in density, as determined by the use of a Kober nephelometer, to a suspension of barium sulphate prepared

by combining 1 cc. of a 1-to-100 solution of barium chlorid with 99 cc. of a 1-to-100 solution of sulphuric acid. A practice was made of reading the tests after they had been incubated at 37° C. for a period of from 36 to 48 hours. The degree of agglutination has been indicated (in Table V) by the characters +, P, S, and -. The + sign has been used to indicate complete clumping of the bacteria, P a partial clumping, S a slight trace of agglutination, and the minus sign to indicate no evidence of clumping.

In addition to the bimonthly agglutination and complement-fixation results indicated in the charts of the individual animals, the results of cultural and inoculation tests of milk, placentas, fetuses, uterine fluids, etc., are also recorded.

#### OBSERVATIONS RELATIVE TO IMMUNITY IN ANIMALS WHICH HAD PREVIOUSLY ABORTED

At the time this experimental work was undertaken there appeared to be a difference of opinion as to the theory of living organism vaccination. The use of massive doses of *Bacterium abortus* created the impression that saturation of the subject with abortion bacteria was the object desired. Under these conditions the prevention of abortion losses was attributed more to the development of a tolerance to abortion bacteria than to resistance to their invasion. This conception appeared to be justified to some degree in view of the fact that little distinction was made between infected and noninfected animals as proper subjects for immunization. In other words, the tendency was to assume that the noninfected animal could be rendered resistant to the infection by the subcutaneous administration of massive doses of the organisms, and that the *Bacterium abortus* udder-infected animal likewise derived resistance of a similar character by contributions to the infection borne at the time of treatment and possibly for months or years previously. Whether or not injections of biological products have a tendency to overcome infection of the udder with abortion bacteria is a problem that has received very little attention. In the absence of evidence to support such a contention it is difficult to conceive logically how the same beneficial results can be reasonably anticipated from the treatment of both types of animals.

Studies that were made of 12 cows, all of which had previously aborted, and from the fetuses, placentas, or uterine fluids of which *Bacterium abortus* had been isolated, furnished information that has some bearing on this matter. All of these animals were eventually successfully bred. Their milk was then tested for the presence or absence of *Bacterium abortus* by guinea-pig inoculations. It was determined that the milk produced by one-third of the number contained abortion bacteria. The milk from eight of the group failed to produce abortion disease in any of the pigs that were inoculated.

Subsequently to collecting the milk samples, 11 of the 12 cows received by the mouth variable amounts of fetal-stomach contents containing *Bacterium abortus*. One cow received one administration, 4 received the material on two occasions, and 6 on three different dates. Cultural tests which were made of the exposure material in all cases indicated the presence of viable abortion bacteria in an uncontaminated condition. Ten living calves were produced by this group. Failure was experienced at time of parturition in isolating *Bacterium abortus* from the placentas or uterine fluids of eight of the number by cultural means or guinea-pig inoculations. The animals having uteri free from *Bacterium abortus* at time of parturition, as indicated by the above-mentioned tests, were those that from the earlier milk tests gave evidence of having overcome the infection in their bodies.

*Bacterium abortus* was isolated from the fetuses, placentas, or uterine fluids of the remaining four cows, despite the fact that animal No. 413 was not subjected to ingestion exposure during the present pregnant period and two of the number (413 and 460) aborted. All four were shown to have been carriers of *Bacterium abortus* infection in their udders during the early months of pregnancy. (See Table II.)

TABLE II.—Exposure tests of aborting cows to determine the degree of immunity resulting from *Bacterium abortus* infection

No. of animal	Date of previous abortion	Date of breeding	Date milk was obtained for inoculation purposes	Results of milk examinations for <i>Bact. abortus</i>	Date of exposure	Exposure material (fetal-stomach contents)		Gestation period	Outcome of pregnancy	Results of uterine examination for <i>Bact. abortus</i>
						Case No.	Amount			
422	Oct. 28, 1917	Feb. 12, 1918 Mar. 22, 1918 May 21, 1918 June 24, 1918 July 17, 1918	Aug. 8, 1918	Negative	Nov. 13, 1918	74	Cc. 50	281 Days	Living calf	Negative.
407	Nov. 2, 1917	Mar. 13, 1918 Feb. 20, 1918	Aug. 14, 1918	do.	Aug. 20, 1918 Nov. 19, 1918	70 74	50 5	288 287	do. do.	Do. Do.
454	Nov. 19, 1917	Feb. 20, 1918 Mar. 20, 1918 May 21, 1918 June 2, 1918 Apr. 13, 1918	Aug. 14, 1918	do.	Aug. 20, 1918 Nov. 19, 1918	70 74	50 5	287	do.	Do.
316	Nov. 18, 1917	Nov. 18, 1918	Aug. 9, 1918	do.	Aug. 20, 1918 Nov. 19, 1918 Feb. 24, 1919	70 74 80	50 5 25	285	do.	Do.
405	May 13, 1917	Jan. 17, 1919	Feb. 24, 1919	do.	May 23, 1919 Feb. 24, 1919 Mar. 19, 1919 May 23, 1919	92 80 88 92	60 60 25 50	280	do.	Do.
450	Apr. 14, 1918	Oct. 10, 1918	Feb. 24, 1919	do.	Feb. 24, 1919 Mar. 19, 1919 May 23, 1919 Feb. 24, 1919 Mar. 19, 1919	92 80 88 92 88	60 60 25 50 25	282	do.	Do.
317	Dec. 18, 1916	Oct. 21, 1918	Aug. 9, 1918	do.	Feb. 24, 1919 Mar. 19, 1919 May 23, 1919 Feb. 24, 1919 Mar. 19, 1919	92 80 88 92 88	60 60 25 50 25	282	do.	Do.
446	Nov. 2, 1917	Mar. 9, 1918	Aug. 8, 1918	Positive	Aug. 20, 1918 May 23, 1919 Nov. 19, 1918 Feb. 24, 1919 Mar. 19, 1919	70 74 70 88 92	50 5 25 50 25	288	do.	Positive.
456	Sept. 3, 1918	Dec. 18, 1918	Feb. 24, 1919	do.	Feb. 24, 1919 Mar. 19, 1919 May 23, 1919 Feb. 24, 1919 Mar. 19, 1919	70 74 88 92 88	60 60 25 50 25	285	do.	Do.
460	Mar. 18, 1918	June 5, 1918 Sept. 9, 1918 Dec. 21, 1918 Mar. 15, 1918 Apr. 13, 1918 May 13, 1918 June 26, 1918 July 20, 1918 Aug. 9, 1918 Aug. 28, 1918 Nov. 2, 1918	Feb. 24, 1919	do.	May 23, 1919 Feb. 24, 1919 Mar. 19, 1919 May 23, 1919 Feb. 24, 1919 Mar. 19, 1919 May 23, 1919	92 88 92 88 92 88 92	50 25 50 25 50 25 50	167	Abortion	Do.
413	Dec. 1, 1917		May 19, 1919	do.	May 23, 1919	92		223	do.	Do.



The manner in which the pregnancies of these 12 animals with an abortion history terminated is regarded as being of particular interest, since it not only indicates that animals may derive a marked degree of immunity as a result of having sustained an attack of the disease, but suggests as well that it may be essential that centers of *Bacterium abortus* infection within the animal shall have ceased to exist before such immunity may readily be demonstrated.

While the fetus, placenta, and uterine exudate expelled by cow No. 413 during 1917 were heavily saturated with abortion bacteria, and from the numerous milk examinations made it could reasonably be concluded that her udder carried the infection continuously until aborting the second time, during June, 1919, little benefit in the way of tolerance to the organism appears to have been acquired. When the manner in which the pregnancy of this animal as well as of the 3 other udder-infected cows used in the exposure experiment terminated is compared with the 8 that gave evidence of having overcome the infection in their bodies, it seems reasonable to infer that the pregnant periods of carriers may well be regarded as speculative so long as the infection persists, and that its total elimination may be necessary before a dependable and true immunity is established. This feature is presumably responsible to a great degree for the differing views held as to whether immunity is derived from an attack of the disease.

#### DISCUSSION OF IMMUNIZATION RESULTS

Considerable encouragement was derived from the results obtained with living abortion organisms under the experimental conditions described. It was gratifying to observe that although marked serological reactions were produced in susceptible heifers by the injections, they became much reduced in intensity or disappeared in a comparatively brief period. This feature was regarded as being of especial interest, inasmuch as what has heretofore appeared to be a logical argument against the use of living organisms has been that heifers so treated may thereafter be rendered spreaders of the disease in herds into which they may be subsequently introduced. The entire absence of sterility troubles and freedom from calving complications in this group of vaccinated subjects constituted further grounds for optimism relative to the procedure, and the simplicity of the immunizing method made it appear especially attractive.

It was only when experimental animals with functioning udders were vaccinated that a certain amount of danger of producing carriers of the disease by the treatment, and of possible defeat of the object desired, was definitely determined. A limited amount of experimental work has been directed toward determining with what frequency the organisms localize in the udders of vaccinated nonpregnant cows. Despite the fact that the percentage of such cases was relatively high in the few mature animals employed in the original experimental work, additional data pertaining to this feature have failed to indicate that as much cause for alarm exists as was suggested by the earlier experiments.

Twenty cows from a herd regarded as being free from infectious abortion were introduced into a herd in which severe abortion losses were being experienced and where 75 per cent of the animals gave positive reactions to the agglutination test for the disease. These 20 animals gave negative results to the agglutination test for infectious abortion at the time of purchase. On November 2, 1921, which was shortly after the introduction of the group, they were injected subcutaneously with 20 cc. of abortion vaccine. On March 6, 1922, samples of blood and milk were taken from those treated and were subjected to the agglutination test. In those cases where 0.005 cc. or lesser amounts of the blood serum caused any agglutination, or where agglutination of the milk occurred even in 0.04 cc. or lesser amounts, guinea-pig inoculations were made with samples of the milk sediment. While 9 groups of guinea pigs consisting of 3 each were injected with sediment

from the milk samples, in no instance could the presence of abortion disease be demonstrated in the guinea pigs when they were destroyed, April 12, 1922, although serological and cultural tests were conducted.

Another group of 11 cows that gave negative results to the agglutination test was vaccinated November 17, 1921. Samples of blood and milk were obtained from these animals June 24, 1922, and tested by the agglutination method. In no case did any of the milk samples show agglutination with 0.04 cc. or lesser amounts. The blood serum of 3 animals showed slight clumping in 0.01 cc. quantities. The serum reactions of the remaining 8 were either negative or showed a titer of less than 0.01 cc. Two guinea pigs were injected with 4 cc. of milk from each cow of this group. When destroyed, two months later, all the pigs yielded negative serological and cultural results for *Bacterium abortus*.

When consideration is given to the results obtained with the 23 animals that were used in a purely experimental capacity, it seems important to bear in mind that so far as could be determined they were free from *Bacterium abortus* infection and susceptible to the disease, and, furthermore, that the vaccination procedure gave indication of being successful only when temporary infection of the animal was occasioned by the treatment. Our knowledge of immunity with reference to other diseases enables us to conceive how under these conditions the administration of abortion vaccine may constitute a reasonable and rational immunizing procedure.

Abortion bacterin failed to show highly encouraging results in this experiment. Although 2 of the 4 animals utilized successfully withstood *Bacterium abortus* exposure that caused controls to abort, it should be borne in mind that this product was used under what may be regarded as ideal conditions; that is, noninfected heifers were used to test the efficacy of the method, and repeated injections of generous amounts were made not only previously to exposure, but in the case of one aborter 4 times previously to conception. Indication that the immunity derived from the bacterin treatment was of but low degree and transient is evidenced by the fact that cow No. 401 acquired *Bacterium abortus* under infection following her second ingestion exposure. Although failure was experienced in demonstrating that bacterin-treated animal 423 was infected with *Bacterium abortus* following her second exposure, the character of her serological reactions indicated that such may have been the case.

#### VACCINE TREATMENT UNDER HERD CONDITIONS

Abortion vaccine has been used in a number of infected herds with the object of acquiring further information as to the practical value of such a product in combating the malady. The interpretation of results under these conditions has not been wholly satisfactory, since, as has been previously mentioned, the matter of *Bacterium abortus* exposure could be controlled only in a measure, and because it has seldom been possible to conduct the necessary bacteriological work during such investigations to permit of differentiating between abortions of *Bacterium abortus* causation and those induced by other factors. Brief consideration, however, will be given to one herd consisting of more than 1,500 head of dairy stock, in which the disease had been prevalent for a number of years, and in which the vaccine was used for the treatment of the nonpregnant animals.

As animals in this herd became available for treatment blood samples were obtained for the application of the agglutination test. Available animals included open cows that had freshened at least two weeks previously and heifers that were within two or three months of breeding age. Two-thirds of the subjects from which blood samples were taken, which group included positive and atypical reactors as well as negative animals, thereupon received a subcutaneous injection of 20 cc. of vaccine, the remaining one-third being employed as controls.

The results derived from the utilization of 1,141 animals under herd conditions are indicated in Tables III and IV.

TABLE III.—Results of vaccine treatment in a *Bacterium abortus* infected dairy herd

## VACCINATED ANIMALS

First period of gestation										Second period of gestation							
Original No.	Sold or died	Conceived		Sterile		Pro-duced living calves		Aborted		Sold or died subsequently to first pregnancy	Calved or aborted a second time	Pro-duced living calves		Aborted		Number of animals with incomplete record of second gestation period <sup>a</sup>	
		No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent			No.	Per cent	No.	Per cent		
772-----	91	617	90.6	64	9.4	536	86.9	81	13.1	128	311	279	89.7	32	10.3	178	

## CONTROLS

369-----	46	294	91.0	29	9.0	242	82.3	52	17.7	63	142	122	85.9	20	14.1			89
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<sup>a</sup> Circumstances necessitated the discontinuance of this experiment before second gestation periods of a considerable number of animals terminated.

TABLE IV.—Grouping of animals shown in Table III with regard to their agglutination reactions

## HEIFERS WHICH GAVE NEGATIVE AGGLUTINATION REACTIONS JUST PRIOR TO VACCINATION

First period of gestation										Second period of gestation						
Original No.	Sold or died	Conceived		Sterile		Pro-duced living calves		Aborted		Sold or died subsequently to first pregnancy	Calved or aborted a second time	Pro-duced living calves		Aborted		Number of animals with incomplete record of second gestation period <sup>a</sup>
		No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent			No.	Per cent	No.	Per cent	
266-----	23	229	94.2	14	5.8	211	92.1	18	7.9	53	86	76	88.4	10	11.6	90

## HEIFERS WHICH GAVE NEGATIVE AGGLUTINATION REACTIONS JUST PRIOR TO USE AS CONTROLS

130-----	17	110	97.3	3	2.7	95	86.4	15	13.6	27	39	32	82.0	7	18.0			44
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## COWS WHICH GAVE NEGATIVE AGGLUTINATION REACTIONS JUST PRIOR TO VACCINATION

292-----	42	234	93.6	16	6.4	210	89.7	24	10.3	50	144	131	91.0	13	9.0			40
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## COWS WHICH GAVE NEGATIVE AGGLUTINATION REACTIONS JUST PRIOR TO USE AS CONTROLS

128-----	23	97	92.4	8	7.6	84	86.6	13	13.4	17	60	55	91.7	5	8.3			20
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## COWS AND HEIFERS WHICH GAVE POSITIVE OR ATYPICAL AGGLUTINATION REACTIONS JUST PRIOR TO VACCINATION

214-----	26	154	82.0	34	18.0	115	74.7	39	25.3	25	81	72	88.9	9	11.1			48
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## COWS AND HEIFERS WHICH GAVE POSITIVE OR ATYPICAL AGGLUTINATION REACTIONS JUST PRIOR TO USE AS CONTROLS

111-----	6	87	82.9	18	17.1	63	72.4	24	27.6	19	43	35	81.4	8	18.6			25
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<sup>a</sup> Circumstances necessitated the discontinuance of this experiment before second gestation periods of a considerable number of animals terminated.

In this herd 772 vaccinated animals are contrasted with 369 controls. During the first gestation period 13.1 per cent of those treated which conceived aborted, whereas 17.6 per cent of the successfully bred controls expelled dead calves. During the second pregnant period, when records were kept of 311 of the vaccinated subjects and 142 controls, 10.2 per cent of the treated animals aborted as against 14 per cent of the controls. The vaccinated animals received no immunizing treatment between the two gestation periods.

In Table IV these 1,141 animals are grouped in such a manner as to give the results of the treatment of heifers and cows that gave negative agglutination reactions at the time they were utilized and animals that gave positive and atypical reactions. Reactions were designated atypical when clumping of 1 cc. of a *Bacterium abortus* suspension was caused by 0.02 cc. or 0.04 cc. of blood serum but not by lesser quantities.

Analysis of the figures indicates that the negative heifers derived the greatest amount of benefit from the treatment. The treated heifers aborted at the rate of 7.9 per cent during the first period and 11.6 per cent during the second, whereas the rate of abortions in the controls was 13.6 per cent during the first period and 18 per cent during the second.

Treated negative cows aborted at a somewhat lower rate than the controls during the first period but at slightly higher rate during the second.

The abortion rate in the positive and atypical group was exceedingly high during the first period. The vaccination of this group gave indication of having caused very slight if any improvement in conditions.

It may be observed by reference to Tables III and IV that when the experimental work in the herd was discontinued sufficient time had not elapsed to permit of obtaining complete data on the second gestation periods, subsequent to vaccination, of all animals. The percentages of animals which aborted and of those which produced living calves during this pregnancy are based upon the performance only of those with a second complete breeding history subsequent to their experimental use.

The benefit derived from the use of the vaccine in this herd, even from the treatment of negative heifers, was somewhat less pronounced than was anticipated in view of the encouraging results obtained by the same method of immunization when negative heifers were subjected artificially to definite amounts of *Bacterium abortus* exposure following vaccination. It was suspected that in this herd factors other than *Bacterium abortus* infection might have been responsible in no small degree for the abortion losses sustained, whereupon blood samples for the application of the agglutination test were taken from 47 animals that aborted after being utilized in the experiment. Thirty of these were included in the vaccinated and 17 in the control group. The 30 aborters in the vaccinated group gave reactions to the agglutination test as follows: Seven positive, 1 atypical, and 22 negative. In the control group there were 10 positive and 7 negative.

The average length of period that intervened between the dates of aborting and the second test was 9½ months. Nineteen of the 47 aborters that were tested by the agglutination method in less than 4 months following the abortion date gave reactions as follows: Eight, or 42.1 per cent positive, and 11, or 57.8 per cent, negative. The remainder of the aborting group, which consisted of 28 animals that were tested between 4 and 23 months after aborting, gave reactions as follows: Ten, or 35.7 per cent, positive, and 18, or 64.2 per cent, negative. While it is possible that the results of the second tests might have differed to a certain extent had they been made in all cases at or near the time the abortions occurred, the percentage of aborters in the two groups, that is, those tested shortly following aborting and those after a more prolonged interval that gave

positive serological reactions, does not differ so greatly as to suggest that the results of the tests were greatly misleading.

When it is taken into consideration that the agglutination results obtained indicated that approximately 60 per cent of these abortions were induced by factors other than *Bacterium abortus* infection, the cause for failure on the part of the vaccine in this herd to yield results more in harmony with those obtained with animals that were subjected to artificial *Bacterium abortus* exposure becomes more readily apparent. These results emphasize the danger of drawing erroneous conclusions as to the value of immunizing agents in infectious abortion unless efforts be made to distinguish between abortions of *Bacterium abortus* and other types of causation.

By referring to the results obtained in the experimental group of 23 animals as indicated in Table I, it will be seen that the administration of fetal-stomach contents containing *Bacterium abortus* proved to be a very effective means of transmitting the disease to susceptible animals, as all the controls in the group, 8 in number, were thus infected. In exposure experiments by different investigators who have used *Bacterium abortus* cultures the percentage of positive results has frequently not been so great. It has therefore seemed reasonable to suspect either that artificial cultivation of the organism causes a considerable reduction in its virulence or that substances of the character of fetal-stomach contents, uterine exudates, etc., that contain the infection may act as a protective envelope for the microorganisms in the alimentary canal until invasion of the tissues occurs.

The amount of material administered to most of the susceptible control animals was presumably greatly in excess of the quantity necessary to cause their infection, as is evidenced by the fact that 5 cc., 22 cc., and 30 cc. resulted in a manner similar to a 60-cc. dosage.

Viscous uterine exudates heavily saturated with *Bacterium abortus* have given indication of possessing infective properties similar to that of *Bacterium abortus* infected fetal-stomach contents when administered by way of mouth, whereas milk in 500-cc. quantities from cows with *Bacterium abortus* infected udders has failed to infect when administered in a similar manner.

When it is taken into consideration that a single feeding of as small a quantity as 5 cc. of fetal-stomach contents proved to be sufficient to produce the disease in a susceptible heifer, then it is reasonably obvious why the malady is often rapidly disseminated in susceptible herds following exposure to infected subjects discharging *Bacterium abortus*.

The length of the period that intervened between dates of ingestion exposure of the susceptible control subjects and the dates when agglutinins or complement-fixing bodies could be demonstrated is of practical interest, since it gives some idea as to how much confidence should be placed in the serological tests for detecting cases of recent infection. This period in the 7 control animals that were tested at bimonthly intervals showed considerable variation. The tests, moreover, in some cases indicated the presence of specific agglutinins soon after their ingestion exposure and their partial disappearance for a period when their presence in increasing amount could again be demonstrated. Two heifers (No. 413 and 446) remained negative to the agglutination test for approximately 4 months following exposure, and heifer No. 460 for 3½ months. A 6 weeks' interval elapsed before serological evidence of infection was obtained in the case of heifer No. 450. Blood serum from animals No. 422, 453, and 470 showed agglutinating properties in less than a month following exposure. The average length of time required for the demonstration of agglutinins was a trifle more than 2 months. Somewhat more time was necessary for the detection of complement-fixing bodies than of agglutinins, a feature which has been observed in other diseases where both methods of diagnosis are used.

## CONCLUSIONS

The data derived from the experimental procedure described seem to justify the following conclusions:

1. That it is possible to demonstrate in cattle an immunity to *Bacterium abortus* infection.
2. That such immunity may be demonstrated both in cows that have sustained an attack of the malady, but give evidence of having overcome the infection in their bodies, and in susceptible animals that have been subjected, previously to conception, to the subcutaneous administration of viable abortion bacteria.
3. That whatever immunity may be engendered by the treatment of animals with abortion bacterin, even when administered in large and frequent dosages both preceding and following conception, is limited and transitory in character.
4. That the vaccination of abortion-infected animals, as well as of those which have overcome the infection, is in all probability an illogical and valueless procedure.
5. That the vaccination of nonpregnant heifers near breeding age with suspensions made from strains of *Bacterium abortus* that have been under artificial cultivation for a year or longer appears seldom to result in the permanent infection of such animals.
6. That the possibility of causing permanent infection is greater when the vaccinated animals have functioning udders.
7. That susceptible heifers may occasionally remain negative to the serological tests for this disease for a period of at least four months after *Bacterium abortus* exposure of such severity as to result subsequently in abortions.
8. That through the ingestion of fetal-stomach material containing *Bacterium abortus* susceptible pregnant animals may readily become infected with the disease.

TABLE V.—Detailed records of individual experimental animals <sup>a</sup>

## VACCINATED HEIFER NO. 402

Serological results												Remarks
Date	Agglutination						Complement fixation					
	0.04	0.02	0.01	0.005	0.002	0.001	0.04	0.02	0.01	0.005	0.002	
Feb. 28, 1917	—	—	—	—	—	—	—	—	—	—	—	Mar. 2, 1917, subcutaneous injection of 20 cc. abortion vaccine.
Mar. 7, 1917	+	—	—	—	—	—	—	—	—	—	—	
Mar. 21, 1917	+	+	+	+	P	P	+	+	+	S	—	Apr. 27, 1917, bred.
Apr. 5, 1917	+	+	+	+	S	—	+	+	+	—	—	
Apr. 21, 1917	+	+	+	+	—	—	+	+	+	—	—	
May 5, 1917	+	+	P	S	—	—	+	+	—	—	—	
May 17, 1917	+	P	S	—	—	—	+	S	—	—	—	
June 1, 1917	+	P	S	—	—	—	P	S	—	—	—	
June 16, 1917	P	P	S	—	—	—	P	—	—	—	—	
June 29, 1917	P	S	—	—	—	—	P	—	—	—	—	
July 13, 1917	S	S	—	—	—	—	—	—	—	—	—	
July 27, 1917	P	S	—	—	—	—	—	—	—	—	—	
Aug. 11, 1917	S	S	—	—	—	—	—	—	—	—	—	Nov. 2, 1917, received 5 cc. fetal-stomach contents (case 42).
Aug. 26, 1917	P	—	—	—	—	—	—	—	—	—	—	
Sept. 7, 1917	S	—	—	—	—	—	—	—	—	—	—	
Sept. 21, 1917	—	—	—	—	—	—	—	—	—	—	—	
Oct. 4, 1917	—	—	—	—	—	—	—	—	—	—	—	
Oct. 18, 1917	S	—	—	—	—	—	—	—	—	—	—	
Nov. 1, 1917	S	—	—	—	—	—	—	—	—	—	—	
Nov. 14, 1917	S	—	—	—	—	—	—	—	—	—	—	
Nov. 28, 1917	P	—	—	—	—	—	—	—	—	—	—	
Dec. 13, 1917	S	—	—	—	—	—	—	—	—	—	—	
Jan. 4, 1918	P	—	—	—	—	—	—	—	—	—	—	

<sup>a</sup> Explanation of symbols: The plus sign indicates complete clumping of bacteria; P, partial clumping; S, slight trace of agglutination; minus sign, no evidence of clumping.

NOTE.—Figures at head of columns indicate cubic centimeters.

TABLE V.—Detailed records of individual experimental animals—Continued

## VACCINATED HEIFER NO. 402—Continued

Serological results												Remarks	
Date	Agglutination						Complement fixation						
	0.04	0.02	0.01	0.005	0.002	0.001	0.04	0.02	0.01	0.005	0.002		
Jan. 18, 1918	P	S	—	—	—	—	—	—	—	—	—	—	Jan. 28, 1918, expelled a living calf. Four guinea pigs inoculated with an emulsion of placenta. All 4 pigs when destroyed, Mar. 9, 1919, gave negative results to agglutination test and negative cultural results for <i>Bact. abortus</i> .
Feb. 2, 1918	P	—	—	—	—	—	—	—	—	—	—	—	
Feb. 16, 1918	—	—	—	—	—	—	—	—	—	—	—	—	
Mar. 1, 1918	S	S	—	—	—	—	—	—	—	—	—	—	
Mar. 16, 1918	S	S	—	—	—	—	—	—	—	—	—	—	
Mar. 30, 1918	—	—	—	—	—	—	—	—	—	—	—	—	
Apr. 13, 1918	S	—	—	—	—	—	—	—	—	—	—	—	
Apr. 29, 1918	P	—	—	—	—	—	—	—	—	—	—	—	
May 10, 1918	—	—	—	—	—	—	—	—	—	—	—	—	
May 25, 1918	—	—	—	—	—	—	—	—	—	—	—	—	
June 8, 1918	P	—	—	—	—	—	—	—	—	—	—	—	
June 21, 1918	—	—	—	—	—	—	—	—	—	—	—	—	
July 6, 1918	—	—	—	—	—	—	—	—	—	—	—	—	
July 19, 1918	—	—	—	—	—	—	—	—	—	—	—	—	
Aug. 1, 1918	P	S	—	—	—	—	—	—	—	—	—	—	
Aug. 16, 1918	P	S	—	—	—	—	—	—	—	—	—	—	
Aug. 30, 1918	P	S	—	—	—	—	—	—	—	—	—	—	Aug. 29, 1918, bred.
Sept. 14, 1918	S	—	—	—	—	—	—	—	—	—	—	—	
Sept. 28, 1918	P	S	—	—	—	—	—	—	—	—	—	—	
Oct. 12, 1918	S	—	—	—	—	—	—	—	—	—	—	—	
Oct. 25, 1918	S	—	—	—	—	—	—	—	—	—	—	—	
Nov. 8, 1918	—	—	—	—	—	—	—	—	—	—	—	—	Nov. 13, 1918, received 50 cc. fetal-stomach contents (case 74).
Nov. 22, 1918	—	—	—	—	—	—	—	—	—	—	—	—	
Dec. 6, 1918	—	—	—	—	—	—	—	—	—	—	—	—	
Dec. 20, 1918	P	—	—	—	—	—	—	—	—	—	—	—	
Jan. 8, 1919	—	—	—	—	—	—	—	—	—	—	—	—	
Jan. 22, 1919	S	—	—	—	—	—	—	—	—	—	—	—	
Feb. 7, 1919	P	S	—	—	—	—	—	—	—	—	—	—	
Feb. 21, 1919	—	—	—	—	—	—	—	—	—	—	—	—	
Mar. 7, 1919	—	—	—	—	—	—	—	—	—	—	—	—	
Mar. 21, 1919	P	S	—	—	—	—	—	—	—	—	—	—	
Apr. 4, 1919	P	P	—	—	—	—	—	—	—	—	—	—	
Apr. 19, 1919	P	S	—	—	—	—	—	—	—	—	—	—	
May 3, 1919	P	S	—	—	—	—	—	—	—	—	—	—	
May 17, 1919	P	S	—	—	—	—	—	—	—	—	—	—	
May 29, 1919	P	P	—	—	—	—	—	—	—	—	—	—	June 4, 1919, expelled a living calf. Three guinea pigs inoculated with emulsion of placenta. All 3 pigs, when destroyed, July 9, 1919, gave negative results to agglutination test and negative cultural results for <i>Bact. abortus</i> .
June 14, 1919	P	S	—	—	—	—	—	—	—	—	—	—	
June 25, 1919	P	P	—	—	—	—	—	—	—	—	—	—	
July 10, 1919	P	—	—	—	—	—	—	—	—	—	—	—	
July 25, 1919	S	S	—	—	—	—	—	—	—	—	—	—	
Aug. 9, 1919	P	—	—	—	—	—	—	—	—	—	—	—	
Aug. 23, 1919	S	—	—	—	—	—	—	—	—	—	—	—	July 7, 1919, a composite sample of milk gave a negative agglutination reaction. Four inoculated guinea pigs gave negative cultural results for <i>Bact. abortus</i> and the 3 which were tested gave negative agglutination results.
Sept. 6, 1919	P	S	—	—	—	—	—	—	—	—	—	—	
Sept. 19, 1919	P	—	—	—	—	—	—	—	—	—	—	—	
Oct. 4, 1919	S	—	—	—	—	—	—	—	—	—	—	—	
Oct. 17, 1919	—	—	—	—	—	—	—	—	—	—	—	—	
Oct. 31, 1919	P	P	—	—	—	—	—	—	—	—	—	—	Oct. 3, 1919, bred.
Nov. 14, 1919	P	S	—	—	—	—	—	—	—	—	—	—	
Nov. 29, 1919	—	—	—	—	—	—	—	—	—	—	—	—	
Dec. 13, 1919	P	—	—	—	—	—	—	—	—	—	—	—	
Jan. 3, 1920	—	—	—	—	—	—	—	—	—	—	—	—	
Jan. 27, 1920	—	—	—	—	—	—	—	—	—	—	—	—	
Feb. 13, 1920	—	—	—	—	—	—	—	—	—	—	—	—	Mar. 18, 1920, received 20 cc. fetal-stomach contents (case 110).

TABLE V.—Detailed records of individual experimental animals—Continued

## VACCINATED HEIFER NO. 402—Continued

Serological results												Remarks
Date	Agglutination						Complement fixation					
	0.04	0.02	0.01	0.005	0.002	0.001	0.04	0.02	0.01	0.005	0.002	
Feb. 28, 1920	—	—	—	—	—	—	—	—	—	—	—	May 10, 1920, received 22 cc. fetal-stomach contents (case 126).
Mar. 13, 1920	—	—	—	—	—	—	—	—	—	—	—	
Mar. 27, 1920	—	—	—	—	—	—	—	—	—	—	—	
Apr. 10, 1920	P	S	—	—	—	—	—	—	—	—	—	
Apr. 24, 1920	P	P	—	—	—	—	—	—	—	—	—	
May 7, 1920	P	S	—	—	—	—	—	—	—	—	—	
May 21, 1920	P	—	—	—	—	—	—	—	—	—	—	
June 5, 1920	S	—	—	—	—	—	—	—	—	—	—	
June 18, 1920	P	—	—	—	—	—	—	—	—	—	—	
July 2, 1920	—	—	—	—	—	—	—	—	—	—	—	
July 17, 1920	P	—	—	—	—	—	—	—	—	—	—	July 2, 1920, three guinea pigs injected with milk; when destroyed, Sept. 3, 1920, gave negative cultural results for <i>Bact. abortus</i> and negative agglutination reactions.
July 30, 1920	S	—	—	—	—	—	—	—	—	—	—	
Aug. 13, 1920	S	—	—	—	—	—	—	—	—	—	—	
Aug. 27, 1920	P	—	—	—	—	—	—	—	—	—	—	
Sept. 10, 1920	P	—	—	—	—	—	—	—	—	—	—	<p>July 10, 1920, expelled a living calf. Four guinea pigs inoculated with uterine material, when destroyed, Sept. 13, 1920, gave negative cultural results for <i>Bact. abortus</i> and negative agglutination reactions.</p> <p>Sept. 10, 1920, slaughtered. Uterus cultured, negative for <i>Bact. abortus</i>. Three guinea pigs, inoculated with milk, when destroyed, Nov. 16, 1920, gave negative cultural results for <i>Bact. abortus</i> and negative agglutination results.</p>

## VACCINATED HEIFER NO. 409

Feb. 28, 1917	-	-	-	-	-	-	-	-	-	-	Mar. 2, 1917, subcutaneous injection of 20 cc. abortion vaccine.
Mar. 7, 1917	+	+	S	-	-	-	-	-	-	-	
Mar. 21, 1917	+	+	+	+	+	S	+	+	P	-	Mar. 23, 1917, subcutaneous injection 20 cc. abortion vaccine.
Apr. 5, 1917	+	+	+	+	+	S	+	+	+	+	Apr. 3, 1917, bred.
Apr. 21, 1917	+	+	+	+	+	S	+	+	+	+	
May 5, 1917	+	+	+	+	P	S	+	+	+	P	
May 17, 1917	P	+	+	+	S	-	+	+	+	-	
June 1, 1917	P	+	P	-	-	-	+	+	S	-	June 8, 1917, received 50 cc. fetal-stomach contents (case 21).
June 16, 1917	+	P	S	-	-	-	+	P	-	-	
June 29, 1917	+	+	-	-	-	-	+	-	-	-	
July 13, 1917	P	S	-	-	-	-	-	-	-	-	
July 27, 1917	P	-	-	-	-	-	-	-	-	-	
Aug. 11, 1917	+	P	-	-	-	-	-	-	-	-	
Aug. 26, 1917	P	-	-	-	-	-	-	-	-	-	
Sept. 7, 1917	-	-	-	-	-	-	-	-	-	-	
Sept. 21, 1917	-	-	-	-	-	-	-	-	-	-	
Oct. 4, 1917	-	-	-	-	-	-	-	-	-	-	
Oct. 18, 1917	-	-	-	-	-	-	-	-	-	-	
Nov. 1, 1917	-	-	-	-	-	-	-	-	-	-	
Nov. 14, 1917	-	-	-	-	-	-	-	-	-	-	
Nov. 28, 1917	-	-	-	-	-	-	-	-	-	-	
Dec. 13, 1917	-	-	-	-	-	-	-	-	-	-	
Jan. 4, 1918	-	-	-	-	-	-	-	-	-	-	
Jan. 18, 1918	S	-	-	-	-	-	-	-	-	-	Jan. 10, 1918, expelled a living calf. Cultures from placenta negative for <i>Bact. abortus</i> . Four guinea pigs inoculation with emulsion of placenta. Two died shortly following inoculation and were not cultured. Two pigs which died Mar. 1 and 9, 1918, respectively, were cultured with negative results for <i>Bact. abortus</i> .



TABLE V.—Detailed records of individual experimental animals—Continued

## VACCINATED HEIFER NO. 409—Continued

Serological results												Remarks
Date	Agglutination						Complement fixation					
	0.04	0.02	0.01	0.005	0.002	0.001	0.04	0.02	0.01	0.005	0.002	
Feb. 2, 1918	—	—	—	—	—	—	—	—	—	—	—	July 24, 1918, bred.  Aug. 20, 1918, received 25 cc. fetal-stomach contents (case 70).
Feb. 16, 1918	S	—	—	—	—	—	—	—	—	—	—	
Mar. 1, 1918	S	—	—	—	—	—	—	—	—	—	—	
Mar. 16, 1918	S	—	—	—	—	—	—	—	—	—	—	
Mar. 30, 1918	—	—	—	—	—	—	—	—	—	—	—	
Apr. 13, 1918	S	—	—	—	—	—	—	—	—	—	—	
Apr. 29, 1918	—	—	—	—	—	—	—	—	—	—	—	
May 10, 1918	—	—	—	—	—	—	—	—	—	—	—	
May 25, 1918	—	—	—	—	—	—	—	—	—	—	—	
June 8, 1918	—	—	—	—	—	—	—	—	—	—	—	
June 21, 1918	—	—	—	—	—	—	—	—	—	—	—	
July 6, 1918	—	—	—	—	—	—	—	—	—	—	—	
July 19, 1918	—	—	—	—	—	—	—	—	—	—	—	
Aug. 1, 1918	—	—	—	—	—	—	—	—	—	—	—	
Aug. 16, 1918	—	—	—	—	—	—	—	—	—	—	—	
Aug. 30, 1918	—	—	—	—	—	—	—	—	—	—	—	
Sept. 14, 1918	—	—	—	—	—	—	—	—	—	—	—	
Sept. 28, 1918	S	—	—	—	—	—	—	—	—	—	—	
Oct. 12, 1918	—	—	—	—	—	—	—	—	—	—	—	
Oct. 25, 1918	S	—	—	—	—	—	—	—	—	—	—	
Nov. 8, 1918	—	—	—	—	—	—	—	—	—	—	—	
Nov. 22, 1918	—	—	—	—	—	—	—	—	—	—	—	
Dec. 6, 1918	—	—	—	—	—	—	—	—	—	—	—	
Dec. 20, 1918	—	—	—	—	—	—	—	—	—	—	—	
Jan. 8, 1919	S	—	—	—	—	—	—	—	—	—	—	
Jan. 22, 1919	—	—	—	—	—	—	—	—	—	—	—	
Feb. 7, 1919	—	—	—	—	—	—	—	—	—	—	—	
Feb. 21, 1919	—	—	—	—	—	—	—	—	—	—	—	
Mar. 7, 1919	—	—	—	—	—	—	—	—	—	—	—	
Mar. 21, 1919	P	—	—	—	—	—	—	—	—	—	—	
Apr. 4, 1919	—	—	—	—	—	—	—	—	—	—	—	
Apr. 19, 1919	P	—	—	—	—	—	—	—	—	—	—	
May 3, 1919	S	—	—	—	—	—	—	—	—	—	—	
May 17, 1919	—	—	—	—	—	—	—	—	—	—	—	
May 29, 1919	—	—	—	—	—	—	—	—	—	—	—	
June 14, 1919	S	—	—	—	—	—	—	—	—	—	—	
June 25, 1919	—	—	—	—	—	—	—	—	—	—	—	
June 27, 1919	—	—	—	—	—	—	—	—	—	—	—	
July 10, 1919	—	—	—	—	—	—	—	—	—	—	—	
July 25, 1919	—	—	—	—	—	—	—	—	—	—	—	
Aug. 9, 1919	—	—	—	—	—	—	—	—	—	—	—	
Aug. 23, 1919	—	—	—	—	—	—	—	—	—	—	—	
Sept. 6, 1919	—	—	—	—	—	—	—	—	—	—	—	
Sept. 19, 1919	P	—	—	—	—	—	—	—	—	—	—	
Oct. 4, 1919	—	—	—	—	—	—	—	—	—	—	—	
Oct. 17, 1919	—	—	—	—	—	—	—	—	—	—	—	
Oct. 31, 1919	—	—	—	—	—	—	—	—	—	—	—	
Nov. 14, 1919	—	—	—	—	—	—	—	—	—	—	—	
Nov. 29, 1919	P	—	—	—	—	—	—	—	—	—	—	
Dec. 13, 1919	S	—	—	—	—	—	—	—	—	—	—	
Jan. 3, 1920	S	S	—	—	—	—	—	—	—	—	—	
Jan. 27, 1920	P	—	—	—	—	—	—	—	—	—	—	
Feb. 13, 1920	S	—	—	—	—	—	—	—	—	—	—	
Feb. 28, 1920	P	—	—	—	—	—	—	—	—	—	—	
Mar. 13, 1920	—	—	—	—	—	—	—	—	—	—	—	
Mar. 27, 1920	—	—	—	—	—	—	—	—	—	—	—	
Apr. 10, 1920	—	—	—	—	—	—	—	—	—	—	—	
Apr. 24, 1920	P	—	—	—	—	—	—	—	—	—	—	
May 7, 1920	P	—	—	—	—	—	—	—	—	—	—	
May 21, 1920	P	—	—	—	—	—	—	—	—	—	—	
June 5, 1920	P	S	—	—	—	—	—	—	—	—	—	
June 18, 1920	S	—	—	—	—	—	—	—	—	—	—	
July 2, 1920	P	—	—	—	—	—	—	—	—	—	—	
July 17, 1920	S	—	—	—	—	—	—	—	—	—	—	
May 10, 1920, received 20 cc. fetal-stomach contents (case 126).												

TABLE V.—Detailed records of individual experimental animals—Continued

## VACCINATED HEIFER NO. 409—Continued

Serological results												Remarks
Date	Agglutination						Complement fixation					
	0.04	0.02	0.01	0.005	0.002	0.001	0.04	0.02	0.01	0.005	0.002	
July 30, 1920	P	—	—	—	—	—	—	—	—	—	—	Aug. 4, 1920, expelled a living calf. Four guinea pigs inoculated with emulsion of placenta, when destroyed Sept. 27, 1920, gave negative agglutination reactions and negative cultural results for <i>Bact. abortus</i> .
Aug. 13, 1920	P	—	—	—	—	—	—	—	—	—	—	
Aug. 27, 1920	S	—	—	—	—	—	—	—	—	—	—	
Sept. 10, 1920	S	—	—	—	—	—	—	—	—	—	—	Oct. 10, 1920, destroyed. The uterus, which was cultured, gave negative results for <i>Bact. abortus</i> .

## VACCINATED HEIFER NO. 417

[illegible]

VACCINATED HEIFER NO. 417—Continued

Serological results												Remarks
Date	Agglutination						Complement fixation					
	0.04	0.02	0.01	0.005	0.002	0.001	0.04	0.02	0.01	0.005	0.002	
Dec. 20, 1918	—	—	—	—	—	—	—	—	—	—	—	Apr. 5, 1919, expelled a living calf. Four guinea pigs inoculated with emulsion of placenta. Two pigs died 3 days later. Two pigs when destroyed June 13, 1919, gave negative agglutination reactions and negative cultural results.
Jan. 8, 1919	S	—	—	—	—	—	—	—	—	—	—	
Jan. 22, 1919	S	—	—	—	—	—	—	—	—	—	—	
Feb. 21, 1919	—	—	—	—	—	—	—	—	—	—	—	
Mar. 7, 1919	—	—	—	—	—	—	—	—	—	—	—	Apr. 5, 1919, expelled a living calf. Four guinea pigs inoculated with emulsion of placenta. Two pigs died 3 days later. Two pigs when destroyed June 13, 1919, gave negative agglutination reactions and negative cultural results.
Mar. 21, 1919	P	—	—	—	—	—	—	—	—	—	—	
Apr. 4, 1919	S	—	—	—	—	—	—	—	—	—	—	
Apr. 19, 1919	P	—	—	—	—	—	—	—	—	—	—	
May 3, 1919	S	—	—	—	—	—	—	—	—	—	—	Apr. 5, 1919, expelled a living calf. Four guinea pigs inoculated with emulsion of placenta. Two pigs died 3 days later. Two pigs when destroyed June 13, 1919, gave negative agglutination reactions and negative cultural results.
May 7, 1919	S	S	—	—	—	—	—	—	—	—	—	
May 29, 1919	P	—	—	—	—	—	—	—	—	—	—	
June 14, 1919	P	—	—	—	—	—	—	—	—	—	—	
June 25, 1919	P	S	—	—	—	—	—	—	—	—	—	July 7, 1919, four guinea pigs inoculated with a composite sample of milk. When destroyed Sept. 8, 1919, negative agglutination reactions and negative cultural results for <i>Bact. abortus</i> were obtained.
June 27, 1919	P	S	—	—	—	—	—	—	—	—	—	
July 10, 1919	P	—	—	—	—	—	—	—	—	—	—	
July 25, 1919	S	—	—	—	—	—	—	—	—	—	—	
Aug. 9, 1919	P	S	—	—	—	—	—	—	—	—	—	July 7, 1919, four guinea pigs inoculated with a composite sample of milk. When destroyed Sept. 8, 1919, negative agglutination reactions and negative cultural results for <i>Bact. abortus</i> were obtained.
Aug. 23, 1919	S	—	—	—	—	—	—	—	—	—	—	
Sept. 6, 1919	—	—	—	—	—	—	—	—	—	—	—	
Sept. 19, 1919	—	—	—	—	—	—	—	—	—	—	—	
Oct. 4, 1919	—	—	—	—	—	—	—	—	—	—	—	Oct. 24, 1919, bred.
Oct. 17, 1919	S	—	—	—	—	—	—	—	—	—	—	
Oct. 31, 1919	S	—	—	—	—	—	—	—	—	—	—	
Nov. 14, 1919	P	—	—	—	—	—	—	—	—	—	—	
Nov. 29, 1919	P	—	—	—	—	—	—	—	—	—	—	Oct. 24, 1919, bred.
Dec. 13, 1919	P	—	—	—	—	—	—	—	—	—	—	
Jan. 3, 1920	P	—	—	—	—	—	—	—	—	—	—	
Jan. 27, 1920	S	—	—	—	—	—	—	—	—	—	—	
Feb. 13, 1920	—	—	—	—	—	—	—	—	—	—	—	Mar. 18, 1920, received 20 cc. fetal-stomach contents (case 110).
Feb. 28, 1920	—	—	—	—	—	—	—	—	—	—	—	
Mar. 13, 1920	—	—	—	—	—	—	—	—	—	—	—	
Mar. 27, 1920	—	—	—	—	—	—	—	—	—	—	—	
Apr. 10, 1920	P	S	—	—	—	—	—	—	—	—	—	Mar. 18, 1920, received 20 cc. fetal-stomach contents (case 110).
Apr. 24, 1920	S	S	—	—	—	—	—	—	—	—	—	
May 7, 1920	P	—	—	—	—	—	—	—	—	—	—	
May 21, 1920	P	—	—	—	—	—	—	—	—	—	—	
June 5, 1920	S	S	—	—	—	—	—	—	—	—	—	May 10, 1920, received 22 cc. fetal-stomach contents (case 126).
June 18, 1920	P	—	—	—	—	—	—	—	—	—	—	
July 2, 1920	S	—	—	—	—	—	—	—	—	—	—	
July 17, 1920	S	—	—	—	—	—	—	—	—	—	—	
July 30, 1920	P	—	—	—	—	—	—	—	—	—	—	May 10, 1920, received 22 cc. fetal-stomach contents (case 126).
Aug. 15, 1920	P	—	—	—	—	—	—	—	—	—	—	
Aug. 27, 1920	S	—	—	—	—	—	—	—	—	—	—	
Sept. 10, 1920	S	—	—	—	—	—	—	—	—	—	—	
Sept. 10, 1920	S	—	—	—	—	—	—	—	—	—	—	July 2, 1920, three guinea pigs inoculated with a composite sample of milk, gave negative agglutination reactions and negative cultural results for <i>Bact. abortus</i> when destroyed Sept. 3, 1920.
July 17, 1920	S	—	—	—	—	—	—	—	—	—	—	July 2, 1920, three guinea pigs inoculated with a composite sample of milk, gave negative agglutination reactions and negative cultural results for <i>Bact. abortus</i> when destroyed Sept. 3, 1920.
July 30, 1920	P	—	—	—	—	—	—	—	—	—	—	
Aug. 15, 1920	P	—	—	—	—	—	—	—	—	—	—	
Aug. 27, 1920	S	—	—	—	—	—	—	—	—	—	—	
Sept. 10, 1920	S	—	—	—	—	—	—	—	—	—	—	July 30, 1920, expelled a living calf. Four guinea pigs inoculated with an emulsion of placenta gave negative agglutination reactions and negative cultural results for <i>Bact. abortus</i> when destroyed Sept. 27, 1920.

Serological results												Remarks
Date	Agglutination						Complement fixation					
	0.04	0.02	0.01	0.005	0.002	0.001	0.04	0.02	0.01	0.005	0.002	
Feb. 28, 1917	-	-	-	-	-	-	-	-	-	-	-	Mar. 2, 1917, subcutaneous admin- istration 20 cc. abortion vaccine.
Mar. 7, 1917	+	+	S	-	-	-	-	-	-	-	-	Mar. 23, 1917, subcutaneous admin- istration 20 cc. abortion vaccine.
Mar. 21, 1917	+	+	+	+	+	S	+	+	P	-	-	
Apr. 5, 1917	+	+	+	+	+	S	+	+	+	+	-	May 3, 1917, bred.
Apr. 21, 1917	+	+	+	+	+	S	+	+	+	+	-	
May 5, 1917	+	+	+	P	P	S	+	+	+	+	P	-
May 17, 1917	P	+	+	S	-	-	+	+	+	+	-	-
June 1, 1917	P	+	P	-	-	-	+	+	+	S	-	-
June 16, 1917	+	P	S	-	-	-	+	P	-	-	-	-
June 29, 1917	+	+	-	-	-	-	+	-	-	-	-	-
July 13, 1917	P	S	-	-	-	-	-	-	-	-	-	-
July 27, 1917	P	-	-	-	-	-	-	-	-	-	-	-
Aug. 11, 1917	+	P	-	-	-	-	-	-	-	-	-	-
Aug. 26, 1917	P	-	-	-	-	-	-	-	-	-	-	-
Sept. 7, 1917	-	-	-	-	-	-	-	-	-	-	-	-
Sept. 21, 1917	-	-	-	-	-	-	-	-	-	-	-	-
Oct. 4, 1917	-	-	-	-	-	-	-	-	-	-	-	-
Oct. 18, 1917	-	-	-	-	-	-	-	-	-	-	-	-
Nov. 1, 1917	-	-	-	-	-	-	-	-	-	-	-	-
Nov. 14, 1917	-	-	-	-	-	-	-	-	-	-	-	-
Nov. 28, 1917	-	-	-	-	-	-	-	-	-	-	-	-
Dec. 13, 1917	-	-	-	-	-	-	-	-	-	-	-	-
Jan. 4, 1918	-	-	-	-	-	-	-	-	-	-	-	-
Jan. 18, 1918	S	-	-	-	-	-	-	-	-	-	-	-
Feb. 2, 1918	-	-	-	-	-	-	-	-	-	-	-	Feb. 5, 1918, expelled a living calf. Three guinea pigs inoculated with an emulsion of placenta. One pig died four days later. Two pigs which were slaughtered Mar. 13, 1918, gave negative agglutination reactions and negative cultural results for <i>Bact. abortus</i> .
Feb. 16, 1918	S	-	-	-	-	-	-	-	-	-	-	-
Mar. 1, 1918	S	-	-	-	-	-	-	-	-	-	-	-
Mar. 16, 1918	S	-	-	-	-	-	-	-	-	-	-	-
Mar. 30, 1918	-	-	-	-	-	-	-	-	-	-	-	-
Apr. 13, 1918	S	-	-	-	-	-	-	-	-	-	-	-
Apr. 29, 1918	-	-	-	-	-	-	-	-	-	-	-	-
May 10, 1918	-	-	-	-	-	-	-	-	-	-	-	-
May 25, 1918	-	-	-	-	-	-	-	-	-	-	-	-
June 8, 1918	-	-	-	-	-	-	-	-	-	-	-	-
June 21, 1918	-	-	-	-	-	-	-	-	-	-	-	-
July 6, 1918	-	-	-	-	-	-	-	-	-	-	-	-
July 19, 1918	-	-	-	-	-	-	-	-	-	-	-	July 25, 1918, bred.
Aug. 1, 1918	-	-	-	-	-	-	-	-	-	-	-	-
Aug. 16, 1918	S	-	-	-	-	-	-	-	-	-	-	Aug. 20, 1918, received 25 cc. fetal stomach contents (case 70).
Aug. 30, 1918	-	-	-	-	-	-	-	-	-	-	-	-
Sept. 14, 1918	-	-	-	-	-	-	-	-	-	-	-	-
Sept. 28, 1918	S	-	-	-	-	-	-	-	-	-	-	-
Oct. 12, 1918	-	-	-	-	-	-	-	-	-	-	-	-
Oct. 25, 1918	S	-	-	-	-	-	-	-	-	-	-	-
Nov. 8, 1918	-	-	-	-	-	-	-	-	-	-	-	-
Nov. 22, 1918	-	-	-	-	-	-	-	-	-	-	-	-
Dec. 6, 1918	-	-	-	-	-	-	-	-	-	-	-	-
Dec. 20, 1918	-	-	-	-	-	-	-	-	-	-	-	-
Jan. 6, 1919	S	-	-	-	-	-	-	-	-	-	-	-
Jan. 22, 1919	-	-	-	-	-	-	-	-	-	-	-	-
Feb. 7, 1919	-	-	-	-	-	-	-	-	-	-	-	-
Feb. 21, 1919	-	-	-	-	-	-	-	-	-	-	-	-
Mar. 7, 1919	-	-	-	-	-	-	-	-	-	-	-	-
Mar. 21, 1919	P	-	-	-	-	-	-	-	-	-	-	-
Apr. 4, 1919	-	-	-	-	-	-	-	-	-	-	-	-
Apr. 19, 1919	P	-	-	-	-	-	-	-	-	-	-	-
May 3, 1919	S	-	-	-	-	-	-	-	-	-	-	May 5, 1919, expelled a living calf. Four guinea pigs inoculated with uterine material. When destroyed June 13, 1919, all gave negative agglutination reactions and nega- tive cultural results for <i>Bact.</i> <i>abortus</i> .

VACCINATED HEIFER NO. 418—Continued

VACCINATED HEIFER NO. 419

Feb. 28, 1918	-	-	-	-	-	-	-	-	-	-	Mar. 30, 1918, subcutaneous administration 20 cc. abortion vaccine.
Apr. 13, 1918	+	+	+	+	P	S	+	+	+	-	
Apr. 29, 1918	+	+	+	+	P	-	+	+	P	-	
May 10, 1918	+	+	P	P	-	-	+	+	+	-	
May 25, 1918	+	+	P	P	-	-	+	S	+	-	
June 8, 1918	+	+	P	P	S	-	-	+	-	-	
June 21, 1918	+	P	P	P	-	-	-	-	-	-	
July 6, 1918	P	P	P	S	-	-	-	-	-	-	
July 19, 1918	P	P	-	-	-	-	-	-	-	-	
Aug. 1, 1918	P	P	-	-	-	-	-	-	-	-	

TABLE V.—Detailed records of individual experimental animals—Continued

VACCINATED HEIFER NO. 419—Continued

Serological results													Remarks
Date	Agglutination						Complement fixation						
	0.04	0.02	0.01	0.005	0.002	0.001	0.04	0.02	0.01	0.005	0.002		
Aug. 16, 1918	P	P	-	-	-	-	-	-	-	-	-	-	Dec. 30, 1918, bred.  Feb. 24, 1919, received 60 cc. fetal-stomach contents (case 80). Mar. 19, 1919, received 25 cc. fetal-stomach contents (case 88).  <

## VACCINATED HEIFER NO. 419—Continued

[illegible]

TABLE V.—Detailed records of individual experimental animals—Continued

## VACCINATED HEIFER NO. 427—Continued

Serological results												Remarks
Date.	Agglutination						Complement fixation					
	0.04	0.02	0.01	0.00	50.00	20.00	10.0	40.0	20.0	10.00	50.00	
Apr. 4, 1919	—	—	—	—	—	—	—	—	—	—	—	Apr. 21, 1919, expelled a living calf. Three guinea pigs inoculated with uterine material. When destroyed, June 13, 1919, negative agglutination reactions and negative cultural results for <i>Bact. abortus</i> were obtained.
Apr. 19, 1919	P	—	—	—	—	—	—	—	—	—	—	Apr. 21, 1919, three guinea pigs inoculated with a sample of milk. When destroyed, June 13, 1919, negative agglutination reactions and negative cultural results obtained.
May 3, 1919	P	—	—	—	—	—	—	—	—	—	—	
May 27, 1919	—	—	—	—	—	—	—	—	—	—	—	
May 23, 1919	—	—	—	—	—	—	—	—	—	—	—	
June 14, 1919	P	S	—	—	—	—	—	—	—	—	—	
June 25, 1919	P	S	—	—	—	—	—	—	—	—	—	July 7, 1919, four guinea pigs inoculated with a composite sample of milk. When destroyed, Sept. 8, 1919, negative agglutination reactions and negative cultural results for <i>Bact. abortus</i> obtained.
July 10, 1919	—	—	—	—	—	—	—	—	—	—	—	
July 25, 1919	S	—	—	—	—	—	—	—	—	—	—	
Aug. 9, 1919	P	S	—	—	—	—	—	—	—	—	—	
Aug. 23, 1919	P	S	—	—	—	—	—	—	—	—	—	
Sept. 6, 1919	—	—	—	—	—	—	—	—	—	—	—	Oct. 3, 1919, [bred].
Sept. 19, 1919	—	—	—	—	—	—	—	—	—	—	—	
Oct. 4, 1919	—	—	—	—	—	—	—	—	—	—	—	
Oct. 17, 1919	—	—	—	—	—	—	—	—	—	—	—	
Oct. 31, 1919	—	—	—	—	—	—	—	—	—	—	—	
Nov. 14, 1919	—	—	—	—	—	—	—	—	—	—	—	
Nov. 29, 1919	P	S	—	—	—	—	—	—	—	—	—	
Dec. 13, 1919	—	—	—	—	—	—	—	—	—	—	—	
Jan. 3, 1920	—	—	—	—	—	—	—	—	—	—	—	
Jan. 27, 1920	S	—	—	—	—	—	—	—	—	—	—	
Feb. 13, 1920	—	—	—	—	—	—	—	—	—	—	—	Mar. 18, 1920, received 20 cc. fetal-stomach contents (case 110).
Feb. 28, 1920	—	—	—	—	—	—	—	—	—	—	—	
Mar. 13, 1920	—	—	—	—	—	—	—	—	—	—	—	
Mar. 27, 1920	—	—	—	—	—	—	—	—	—	—	—	
Apr. 10, 1920	P	S	—	—	—	—	—	—	—	—	—	
Apr. 24, 1920	P	S	—	—	—	—	—	—	—	—	—	May 10, 1920, received 22 cc. fetal-stomach contents (case 126).
May 7, 1920	S	S	—	—	—	—	—	—	—	—	—	
May 21, 1920	S	—	—	—	—	—	—	—	—	—	—	
June 5, 1920	—	—	—	—	—	—	—	—	—	—	—	
June 18, 1920	—	—	—	—	—	—	—	—	—	—	—	
July 2, 1920	P	—	—	—	—	—	—	—	—	—	—	July 2, 1920, three guinea pigs inoculated with a composite sample of milk. When destroyed, Sept. 13, 1920, gave negative agglutination reactions and negative cultural results for <i>Bact. abortus</i> .
July 17, 1920	P	—	—	—	—	—	—	—	—	—	—	
July 30, 1920	S	—	—	—	—	—	—	—	—	—	—	
Aug. 13, 1920	—	—	—	—	—	—	—	—	—	—	—	
Aug. 27, 1920	S	—	—	—	—	—	—	—	—	—	—	
July 12, 1920												July 12, 1920, expelled a living calf. Four guinea pigs inoculated with an emulsion of placenta; when destroyed, Sept. 13, 1920, gave negative agglutination reactions and negative cultural results for <i>Bact. abortus</i> .



TABLE V.—*Detailed records of individual experimental animals*—Continued

VACCINATED HEIFER NO. 462

[illegible]

VACCINATED HEIFER NO. 447

Serological results												Remarks
Date	Agglutination						Complement fixation					
	0.04	0.02	0.01	0.005	0.002	0.001	0.04	0.02	0.01	0.005	0.002	
Feb. 28, 1918	-	-	-	-	-	-	-	-	-	-	-	
Mar. 16, 1918	-	-	-	-	-	-	-	-	-	-	-	
Apr. 13, 1918	+	+	+	+	+	P	+	+	+	+	+	
Apr. 29, 1918	+	+	+	+	P	S	+	+	+	+	+	
May 10, 1918	+	+	+	P	P	-	+	+	+	-	-	
May 25, 1918	+	+	P	P	P	-	+	+	P	-	-	
June 8, 1918	+	P	P	-	-	-	+	P	-	-	-	
June 21, 1918	+	P	S	-	-	-	+	S	-	-	-	
July 6, 1918	+	S	S	-	-	-	+	-	-	-	-	July 1, 1918, bred.
July 19, 1918	P	S	S	-	-	-	-	-	-	-	-	
Aug. 1, 1918	P	S	S	-	-	-	-	-	-	-	-	
Aug. 16, 1918	P	P	-	-	-	-	-	-	-	-	-	
Aug. 30, 1918	P	P	-	-	-	-	-	-	-	-	-	
Sept. 14, 1918	P	P	-	-	-	-	-	-	-	-	-	
Sept. 28, 1918	S	S	-	-	-	-	-	-	-	-	-	
Oct. 12, 1918	P	-	-	-	-	-	-	-	-	-	-	
Oct. 25, 1918	S	-	-	-	-	-	-	-	-	-	-	Nov. 13, 1918, received 30 cc. fetal-stomach contents (case 74).
Nov. 8, 1918	S	-	-	-	-	-	-	-	-	-	-	
Nov. 22, 1918	-	-	-	-	-	-	-	-	-	-	-	
Dec. 6, 1918	S	-	-	-	-	-	-	-	-	-	-	
Dec. 20, 1918	P	-	-	-	-	-	-	-	-	-	-	
Jan. 8, 1919	P	S	-	-	-	-	-	-	-	-	-	
Jan. 22, 1919	S	S	-	-	-	-	-	-	-	-	-	
Feb. 7, 1919	P	-	-	-	-	-	-	-	-	-	-	
Feb. 21, 1919	P	P	S	-	-	-	-	-	-	-	-	
Mar. 7, 1919	+	+	+	P	P	-	+	P	-	-	-	Mar. 10, 1919, three guinea pigs inoculated with vaginal material and three with colostrum; when destroyed, June 13, 1919, gave negative agglutination reactions and negative cultural results for <i>Bact. abortus</i> .
Mar. 21, 1919	+	+	+	+	+	+	+	+	+	+	-	
Apr. 4, 1919	+	+	+	+	+	+	+	+	+	+	P	
Apr. 19, 1919	+	+	+	+	+	+	+	+	+	+	+	Mar. 17, 1919, expelled a dead fetus. <i>Bact. abortus</i> isolated from both fetus and placenta by cultural methods.

## VACCINATED COW NO. 408

Feb. 7, 1919	-	-	-	-	-	-	-	-	-	-	-	Feb. 24, 1919, subcutaneous admin- istration 20 cc. abortion vaccine.
Feb. 21, 1916	-	-	-	-	-	-	-	-	-	-	-	
Mar. 7, 1919	+	+	+	+	P	-	+	+	+	-	-	
Mar. 21, 1919	+	+	+	+	P	S	+	+	+	S	-	
Apr. 4, 1919	+	+	+	+	P	-	+	+	+	-	-	
Apr. 19, 1919	+	+	+	+	S	-	+	+	P	-	-	Apr. 12, 1919, bred.
May 3, 1919	+	+	+	+	S	-	+	+	S	-	-	
May 17, 1919	+	+	+	P	-	-	+	+	P	-	-	May 24, 1919, received 50 cc. fetal stomach contents (case 92).
May 29, 1919	+	+	+	S	-	-	+	+	S	-	-	
June 14, 1919	+	+	+	S	-	-	+	+	-	-	-	
June 25, 1919	+	+	+	-	-	-	+	P	-	-	-	
July 10, 1919	+	+	P	-	-	-	+	-	-	-	-	
July 25, 1919	+	+	S	-	-	-	P	-	-	-	-	
Aug. 9, 1919	+	P	S	-	-	-	-	-	-	-	-	
Aug. 20, 1919	+	P	-	-	-	-	-	-	-	-	-	
Sept. 6, 1919	P	S	-	-	-	-	-	-	-	-	-	
Sept. 19, 1919	P	S	-	-	-	-	-	-	-	-	-	
Oct. 4, 1919	P	P	-	-	-	-	-	-	-	-	-	
Oct. 17, 1919	+	P	S	-	-	-	-	-	-	-	-	
Oct. 31, 1919	+	P	P	S	-	-	+	+	P	-	-	
Nov. 4, 1919	P	P	+	+	+	+	+	+	+	P	+	
Nov. 14, 1919	P	P	+	+	+	+	+	+	+	+	+	Nov. 20, 1919, three guinea pigs in- oculated with a composite sample of milk. When destroyed, Jan. 7, 1920, gave positive agglutination reactions. <i>Bact. abortus</i> isolated from all pigs.



TABLE V.—Detailed records of individual experimental animals—Continued

## VACCINATED COW NO. 410—Continued

Serological results												Remarks
Date	Agglutination						Complement fixation					
	0.04	0.02	0.01	0.005	0.002	0.001	0.04	0.02	0.01	0.005	0.002	
Sept. 25, 1920	+	+	+	+	+	+	+	+	+	—	P	Dec. 14, 1920, animal slaughtered. <i>Bact. abortus</i> isolated from uterus and milk by cultural methods.
Oct. 7, 1920	+	+	+	+	+	S	+	+	+	+	S	
Oct. 22, 1920	+	+	+	+	+	+	+	+	+	+	S	
Nov. 8, 1920	+	+	+	+	+	+	+	+	+	+	P	
Nov. 27, 1920	+	+	+	+	+	+	+	+	+	+	P	
Dec. 11, 1920	+	+	+	+	+	+	+	+	+	+	—	

## VACCINATED COW NO. 416

Feb. 13, 1920	—	—	—	—	—	—	—	—	—	—	—	Mar. 2, 1920, subcutaneous administration 20 cc. abortion vaccine. Mar. 20, 1920, four guinea pigs inoculated with milk. Negative cultural results and negative agglutination reactions for <i>Bact. abortus</i> when destroyed.
Feb. 28, 1920	—	—	—	—	—	—	—	—	—	—	—	
Mar. 13, 1920	+	+	P	S	—	—	P	—	—	—	—	
Mar. 27, 1920	+	+	+	+	P	—	+	+	+	—	—	Apr. 15, 1920, four guinea pigs inoculated with milk. Negative cultural results and negative agglutination reactions for <i>Bact. abortus</i> when destroyed, June 5, 1920.
Apr. 10, 1920	+	+	+	+	P	S	+	+	+	P	—	
Apr. 24, 1920	+	+	+	P	P	—	+	+	+	—	—	
May 7, 1920	+	+	+	P	S	—	+	+	P	—	—	May 5, 1920, three guinea pigs inoculated with milk. Negative cultural results and negative agglutination reactions when destroyed, June 17, 1920.
May 21, 1920	+	+	+	P	S	—	+	+	—	—	—	
June 5, 1920	+	+	P	S	—	—	+	P	—	—	—	
June 18, 1920	+	P	S	—	—	—	+	—	—	—	—	May 5, 1920, bred. May 10, 1920, received 22 cc. fetal-stomach contents (case 126). May 17, 1920, three guinea pigs inoculated with milk; when destroyed gave negative cultural results and negative agglutination reactions.
July 2, 1920	+	P	S	—	—	—	—	—	—	—	—	
July 18, 1920	S	S	—	—	—	—	—	—	—	—	—	
July 30, 1920	S	—	—	—	—	—	—	—	—	—	—	July 2, 1920, three guinea pigs inoculated with milk; when destroyed, Sept. 3, 1920, gave negative cultural results and negative agglutination reactions.
Aug. 13, 1920	+	+	P	P	—	—	+	+	—	—	—	
Aug. 27, 1920	+	+	+	+	P	S	+	+	+	+	S	
Sept. 10, 1920	+	+	+	+	+	P	+	+	+	+	+	Aug. 22, 1920, three guinea pigs inoculated with milk; when destroyed, Sept. 13, 1920, gave positive agglutination reactions and positive cultural results for <i>Bact. abortus</i> .
Sept. 25, 1920	+	+	+	+	+	+	+	+	+	+	+	
Oct. 7, 1920	+	+	+	+	+	S	+	+	+	+	—	
Oct. 22, 1920	+	+	+	+	+	+	+	+	+	+	—	Feb. 19, 1921, expelled a living calf. Three guinea pigs inoculated with an emulsion of placenta when destroyed gave negative agglutination reactions and negative cultural results for <i>Bact. abortus</i> .
Nov. 8, 1920	+	+	+	+	+	P	+	+	+	+	S	
Nov. 27, 1920	+	+	+	+	+	—	+	+	+	+	—	
Dec. 11, 1920	+	+	+	+	+	+	+	+	+	+	—	Feb. 28, 1921, <i>Bact. abortus</i> isolated from milk by cultural methods.
Dec. 29, 1920	+	+	+	P	P	—	+	+	+	P	—	
Jan. 20, 1921	+	+	+	P	S	—	+	+	+	—	—	
Feb. 14, 1921	+	+	+	P	S	—	+	+	+	—	—	
Mar. 3, 1921	+	+	+	P	P	—	+	+	+	—	—	
Mar. 17, 1921	+	+	P	P	P	—	+	+	+	—	—	
Apr. 1, 1921	+	+	P	P	S	—	+	+	P	—	—	

TABLE V.—Detailed records of individual experimental animals—Continued

## BACTERIN-TREATED HEIFER NO. 423

Serological results												Remarks
Date	Agglutination						Complement fixation					
	0.04	0.02	0.01	0.005	0.002	0.001	0.04	0.02	0.01	0.005	0.002	
Nov. 3, 1916	—	—	—	—	—	—	—	—	—	—	—	Feb. 1, 1917, subcutaneous administration 20 cc. abortion bacterin. Mar. 2, 1917, subcutaneous administration 20 cc. abortion bacterin. Mar. 23, 1917, bred.
Feb. 2, 1917	—	—	—	—	—	—	—	—	—	—	—	
Feb. 8, 1917	+	+	+	S	—	—	+	+	+	+	S	
Feb. 19, 1917	+	+	+	+	—	—	+	+	+	S	—	Apr. 2, 1917, subcutaneous administration 20 cc. abortion bacterin. May 2, 1917, subcutaneous administration 20 cc. abortion bacterin. June 7, 1917, subcutaneous administration 20 cc. abortion bacterin. June 8, 1917, received 50 cc. fetal-stomach contents (case 21).
Mar. 3, 1917	+	+	+	+	—	—	+	+	+	S	—	
Mar. 14, 1917	+	+	—	—	—	—	+	+	+	—	—	
Mar. 22, 1917	+	+	S	—	—	—	+	+	+	—	—	Apr. 2, 1917, subcutaneous administration 20 cc. abortion bacterin. May 2, 1917, subcutaneous administration 20 cc. abortion bacterin. June 7, 1917, subcutaneous administration 20 cc. abortion bacterin. June 8, 1917, received 50 cc. fetal-stomach contents (case 21).
Apr. 2, 1917	+	P	—	—	—	—	+	+	S	—	—	
Apr. 14, 1917	+	+	P	—	—	—	+	+	+	S	—	
Apr. 23, 1917	P	P	S	—	—	—	+	+	P	—	—	July 2, 1917, subcutaneous administration 20 cc. abortion bacterin. Aug. 4, 1917, subcutaneous administration 20 cc. abortion bacterin. Sept. 4, 1917, subcutaneous administration 20 cc. abortion bacterin. Oct. 5, 1917, subcutaneous administration 20 cc. abortion bacterin.
May 12, 1917	+	+	+	P	S	—	+	+	+	+	—	
June 1, 1917	P	P	P	—	—	—	+	+	P	—	—	
June 16, 1917	+	P	S	—	—	—	+	P	—	—	—	July 2, 1917, subcutaneous administration 20 cc. abortion bacterin. Aug. 4, 1917, subcutaneous administration 20 cc. abortion bacterin. Sept. 4, 1917, subcutaneous administration 20 cc. abortion bacterin. Oct. 5, 1917, subcutaneous administration 20 cc. abortion bacterin.
June 29, 1917	+	P	S	S	—	—	+	+	—	—	—	
July 13, 1917	+	P	S	—	—	—	+	+	+	—	—	
July 27, 1917	+	P	S	—	—	—	+	+	P	—	—	Nov. 3, 1917, subcutaneous administration 20 cc. abortion bacterin. Dec. 3, 1917, subcutaneous administration 20 cc. abortion bacterin. Dec. 30, 1917, expelled a living calf. 6 guinea pigs inoculated with emulsion of placenta. 3 pigs when destroyed, Mar. 2, 1918, gave negative agglutination reactions and negative cultural results for <i>Bact. abortus</i> . 3 pigs which died gave negative cultural results.
Aug. 11, 1917	+	+	+	P	—	—	+	+	+	P	—	
Aug. 26, 1917	+	+	P	P	—	—	+	+	S	—	—	
Sept. 7, 1917	+	P	S	—	—	—	+	+	+	P	—	Nov. 3, 1917, subcutaneous administration 20 cc. abortion bacterin. Dec. 3, 1917, subcutaneous administration 20 cc. abortion bacterin. Dec. 30, 1917, expelled a living calf. 6 guinea pigs inoculated with emulsion of placenta. 3 pigs when destroyed, Mar. 2, 1918, gave negative agglutination reactions and negative cultural results for <i>Bact. abortus</i> . 3 pigs which died gave negative cultural results.
Sept. 21, 1917	+	S	S	—	—	—	+	+	+	—	—	
Oct. 4, 1917	+	—	—	—	—	—	+	+	+	—	—	
Oct. 18, 1917	+	+	P	P	—	—	+	+	+	S	S	Nov. 3, 1917, subcutaneous administration 20 cc. abortion bacterin. Dec. 3, 1917, subcutaneous administration 20 cc. abortion bacterin. Dec. 30, 1917, expelled a living calf. 6 guinea pigs inoculated with emulsion of placenta. 3 pigs when destroyed, Mar. 2, 1918, gave negative agglutination reactions and negative cultural results for <i>Bact. abortus</i> . 3 pigs which died gave negative cultural results.
Nov. 1, 1917	+	+	+	+	—	—	+	+	+	+	—	
Nov. 14, 1917	+	+	P	P	—	—	+	+	+	P	—	
Nov. 28, 1917	+	P	S	S	—	—	+	+	+	S	—	Dec. 3, 1917, subcutaneous administration 20 cc. abortion bacterin. Dec. 30, 1917, expelled a living calf. 6 guinea pigs inoculated with emulsion of placenta. 3 pigs when destroyed, Mar. 2, 1918, gave negative agglutination reactions and negative cultural results for <i>Bact. abortus</i> . 3 pigs which died gave negative cultural results.
Dec. 13, 1917	+	P	S	—	—	—	+	+	+	—	—	
Jan. 4, 1918	P	S	—	—	—	—	+	+	P	—	—	
Jan. 18, 1918	+	+	+	+	P	P	+	+	+	+	—	June 5, 1918, bred.
Feb. 2, 1918	+	+	+	+	P	P	—	+	+	+	—	
Feb. 16, 1918	+	+	+	+	P	P	—	+	+	+	—	
Mar. 1, 1918	+	+	+	+	P	P	—	+	+	+	+	June 5, 1918, bred.
Mar. 16, 1918	+	+	+	+	P	P	—	+	+	+	+	
Mar. 30, 1918	+	+	+	+	P	P	—	+	+	+	+	
Apr. 13, 1918	+	+	+	+	P	P	—	+	+	+	+	June 5, 1918, bred.
Apr. 29, 1918	+	+	+	+	P	P	—	+	+	+	+	
May 10, 1918	+	+	+	+	P	P	—	+	+	+	+	
May 25, 1918	+	+	+	+	P	P	—	+	+	+	+	Aug. 20, 1918, received 25 cc. fetal-stomach contents (case 70).
June 8, 1918	+	+	+	+	P	P	—	+	+	+	+	
June 21, 1918	+	+	+	+	P	P	—	+	+	+	+	
July 6, 1918	+	+	+	+	P	P	—	+	+	+	+	Aug. 20, 1918, received 25 cc. fetal-stomach contents (case 70).
July 19, 1918	+	+	+	+	P	P	—	+	+	+	+	
Aug. 1, 1918	+	+	+	+	P	P	—	+	+	+	+	
Aug. 16, 1918	S	S	—	—	—	—	+	+	+	—	—	Aug. 20, 1918, received 25 cc. fetal-stomach contents (case 70).
Aug. 30, 1918	P	P	S	—	—	—	+	+	+	—	—	
Sept. 14, 1918	P	P	P	—	—	—	+	+	+	—	—	
Sept. 28, 1918	+	+	+	P	P	S	+	+	+	+	+	Aug. 20, 1918, received 25 cc. fetal-stomach contents (case 70).
Oct. 12, 1918	+	+	+	P	P	S	+	+	+	+	+	
Oct. 25, 1918	+	+	+	P	P	S	+	+	+	+	+	
Nov. 8, 1918	+	+	+	P	P	S	+	+	+	+	+	Aug. 20, 1918, received 25 cc. fetal-stomach contents (case 70).
Nov. 22, 1918	+	+	+	P	P	S	+	+	+	+	+	
Dec. 6, 1918	+	+	+	P	P	S	+	+	+	+	+	
Dec. 20, 1918	+	+	+	P	P	S	+	+	+	+	+	Aug. 20, 1918, received 25 cc. fetal-stomach contents (case 70).
Jan. 8, 1919	+	+	+	P	P	S	+	+	+	+	+	
	+	+	+	P	P	S	+	+	+	+	+	

TABLE V.—Detailed records of individual experimental animals—Continued

BACTERIN-TREATED HEIFER NO. 423—Continued

Serological results												Remarks
Date	Agglutination						Complement fixation					
	0.04	0.02	0.01	0.005	0.002	0.001	0.04	0.02	0.01	0.005	0.002	
Jan. 22, 1919	P	+	+	+	P	S	+	+	+	+	+	Jan. 22, 1919, expelled a dead fetus. Failure experienced in isolation of <i>Bact. abortus</i> from fetus. 3 guinea pigs inoculated with an emulsion of placenta succumbed to septic infection.
Feb. 7, 1919	P	P	+	+	P	P	+	+	+	+	+	
Feb. 21, 1919	P	P	+	+	P	S	+	+	+	+	+	
Mar. 7, 1919	+	+	+	+	P	S	+	+	+	+	+	
												Jan. 28, 1919, 4 guinea pigs inoculated with uterine material. When destroyed, Mar. 3, 1919, negative agglutination reactions and negative cultural results for <i>Bact. abortus</i> obtained.
Mar. 21, 1919	P	+	+	+	P	S	+	+	+	+	+	

BACTERIN-TREATED HEIFER NO. 401

Nov. 3, 1916	—	—	—	—	—	—	—	—	—	—	—	Aug. 8, 1917, bred.
Jan. 7, 1918	—	—	—	—	—	—	—	—	—	—	—	
Jan. 18, 1918	+	P	P	S	—	—	+	+	—	—	—	Dec. 17, 1917, subcutaneous administration 20 cc. abortion bacterin.
Feb. 2, 1918	+	+	S	S	—	—	+	+	—	—	—	
Feb. 16, 1918	P	S	—	—	—	—	+	+	—	—	—	Jan. 2, 1918, subcutaneous administration 20 cc. abortion bacterin.
Mar. 1, 1918	+	+	—	—	—	—	+	+	—	—	—	
Mar. 16, 1918	+	+	P	—	—	—	+	+	+	—	—	Jan. 5, 1918, received 30 cc. fetal-stomach contents (case 54).
Mar. 30, 1918	P	S	S	—	—	—	+	+	S	—	—	
Apr. 13, 1918	P	S	S	—	—	—	+	S	—	—	—	Jan. 22, 1918, subcutaneous administration 20 cc. abortion bacterin.
Apr. 29, 1918	+	+	P	P	S	—	+	S	—	—	—	
May 10, 1918	+	P	P	—	—	—	+	+	P	—	—	Feb. 2, 1918, subcutaneous administration 20 cc. abortion bacterin.
May 25, 1918	+	+	+	P	—	—	+	+	+	+	—	
												Mar. 4, 1918, subcutaneous administration 20 cc. abortion bacterin.
June 8, 1918	+	+	+	P	—	—	+	+	+	+	—	
June 21, 1918	+	+	+	P	—	—	+	+	+	+	—	Mar. 25, 1918, subcutaneous administration 20 cc. abortion bacterin.
July 6, 1918	+	+	+	P	S	—	+	+	P	—	—	
July 19, 1918	+	+	P	P	P	—	+	+	P	—	—	Apr. 15, 1918, subcutaneous administration 20 cc. abortion bacterin.
Aug. 1, 1918	+	+	S	—	—	—	+	+	—	—	—	
Aug. 16, 1918	+	+	P	P	—	—	+	+	+	—	—	Mar. 8, 1918, expelled a living calf. 4 guinea pigs inoculated with emulsion of placenta. 2 pigs, destroyed July 24, 1918, gave negative agglutination reactions and negative cultural results for <i>Bact. abortus</i> . Two pigs not cultured.
Aug. 30, 1918	+	+	+	P	—	—	+	+	+	—	—	
Sept. 14, 1918	+	+	+	+	—	—	+	+	+	—	—	Sept. 23, 1918, bred.
Sept. 28, 1918	+	+	P	S	—	—	+	+	P	—	—	
Oct. 12, 1918	+	+	P	S	—	—	+	+	—	—	—	Oct. 14, 1918, bred.
Oct. 25, 1918	+	P	P	S	—	—	+	+	—	—	—	
Nov. 8, 1918	+	P	P	P	—	—	+	+	—	—	—	Nov. 13, 1918, received 20 cc. fetal-stomach contents (case 74).
Nov. 22, 1918	P	P	S	S	—	—	+	P	—	—	—	
Dec. 6, 1918	P	P	S	—	—	—	+	S	—	—	—	Nov. 22, 1919, expelled a dead fetus. Failure experienced in isolation of <i>Bact. abortus</i> from fetus. 3 guinea pigs inoculated with an emulsion of placenta succumbed to septic infection.
Dec. 20, 1918	P	P	P	—	—	—	+	S	—	—	—	
Jan. 8, 1919	+	P	P	S	—	—	+	+	—	—	—	Jan. 28, 1919, 4 guinea pigs inoculated with uterine material. When destroyed, Mar. 3, 1919, negative agglutination reactions and negative cultural results for <i>Bact. abortus</i> obtained.
Jan. 22, 1919	+	P	P	P	S	—	+	+	+	—	—	
Feb. 7, 1919	+	+	P	P	—	—	+	+	+	—	—	Jan. 22, 1919, expelled a dead fetus. Failure experienced in isolation of <i>Bact. abortus</i> from fetus. 3 guinea pigs inoculated with an emulsion of placenta succumbed to septic infection.
Feb. 21, 1919	+	+	+	P	S	—	+	+	+	—	—	
Mar. 7, 1919	+	+	+	+	P	—	+	+	+	+	—	Jan. 28, 1919, 4 guinea pigs inoculated with uterine material. When destroyed, Mar. 3, 1919, negative agglutination reactions and negative cultural results for <i>Bact. abortus</i> obtained.
Mar. 21, 1919	+	+	+	+	+	—	+	+	+	+	P	
Apr. 4, 1919	+	+	+	+	+	—	+	+	+	+	+	Jan. 28, 1919, 4 guinea pigs inoculated with uterine material. When destroyed, Mar. 3, 1919, negative agglutination reactions and negative cultural results for <i>Bact. abortus</i> obtained.
Apr. 19, 1919	+	+	+	+	+	—	+	+	+	+	+	

TABLE V.—Detailed records of individual experimental animals—Continued

## BACTERIN-TREATED HEIFER NO. 401—Continued

Serological results												Remarks
Date	Agglutination						Complement fixation					
	0.04	0.02	0.01	0.005	0.002	0.001	0.04	0.02	0.01	0.005	0.002	
May 3, 1919	+	+	+	+	+	+	+	+	+	+	+	May 19, 1919, 4 guinea pigs inoculated with a sample of milk; when destroyed, June 30, 1919, gave positive agglutination reactions and positive cultural results for <i>Bact. abortus</i> .
May 17, 1919	+	+	+	+	+	+	+	+	+	+	+	
May 29, 1919	+	+	+	+	+	+	+	+	+	+	+	
June 14, 1919	+	+	+	+	+	+	+	+	+	+	+	
June 25, 1919	+	+	+	+	+	+	+	+	+	+	+	July 16, 1919, 3 guinea pigs inoculated with vaginal mucus. When destroyed, Sept. 9, 1919, negative agglutination reactions and negative cultural results obtained.
July 10, 1919	+	+	+	+	+	+	+	+	+	+	+	
July 25, 1919	+	+	+	+	+	+	+	+	+	+	+	
Aug. 9, 1919	+	+	+	+	+	+	+	+	+	+	+	
Aug. 23, 1919	+	+	+	+	+	+	+	+	+	+	+	July 19, 1919, expelled a living calf. 4 guinea pigs inoculated with emulsion of placenta. Pigs died during following week. July 23, 1919, 4 guinea pigs inoculated with uterine material. When destroyed, Sept. 9, 1919, negative agglutination reactions and negative cultural results obtained.

## BACTERIN-TREATED HEIFER No. 404

Nov. 3, 1916	—	—	—	—	—	—	—	—	—	—	—	Feb. 1, 1917, subcutaneous administration 20 cc. abortion bacterin.
Feb. 2, 1917	—	—	—	—	—	—	—	—	—	—	—	
Feb. 8, 1917	P	P	—	—	—	—	P	—	—	—	—	Mar. 1, 1917, subcutaneous administration 20 cc. abortion bacterin.
Feb. 19, 1917	+	+	P	S	—	—	+	+	—	—	—	
Mar. 3, 1917	+	+	P	—	—	—	+	+	—	—	—	Apr. 2, 1917, subcutaneous administration 20 cc. abortion bacterin.
Mar. 14, 1917	+	P	P	S	—	—	+	P	S	—	—	
Mar. 22, 1917	+	P	S	—	—	—	+	—	—	—	—	May 2, 1917, subcutaneous administration 20 cc. abortion bacterin.
Apr. 3, 1917	+	P	—	—	—	—	+	—	—	—	—	
Apr. 14, 1917	+	+	P	—	—	—	+	+	+	—	—	May 24, 1917, bred.
Apr. 23, 1917	+	P	P	—	—	—	+	+	—	—	—	
May 12, 1917	+	+	+	S	—	—	+	+	+	—	—	June 2, 1917, subcutaneous administration 20 cc. abortion bacterin.
June 1, 1917	+	+	+	P	—	—	+	+	S	S	—	
June 16, 1917	+	+	S	—	—	—	+	+	—	—	—	June 7, 1917, received 50 cc. fetal stomach contents (case 21).
June 29, 1917	+	P	—	—	—	—	+	P	—	—	—	
July 13, 1917	+	P	P	—	—	—	+	+	—	—	—	July 2, 1917, subcutaneous administration 20 cc. abortion bacterin.
July 27, 1917	+	P	S	—	—	—	+	+	—	—	—	
Aug. 11, 1917	+	P	P	S	—	—	+	+	+	—	—	Aug. 4, 1917, subcutaneous administration 20 cc. abortion bacterin.
Aug. 26, 1917	+	+	P	S	—	—	+	+	+	—	—	
Sept. 7, 1917	+	P	P	—	—	—	+	+	+	—	—	Sept. 4, 1917, subcutaneous administration 20 cc. abortion bacterin.
Sept. 21, 1917	+	P	S	—	—	—	+	+	—	—	—	
Oct. 4, 1917	+	+	S	—	—	—	+	+	S	—	—	Oct. 5, 1917, subcutaneous administration 20 cc. abortion bacterin.
Oct. 18, 1917	+	+	+	+	—	—	+	+	+	+	P	
Nov. 1, 1917	+	+	+	+	—	—	+	+	+	+	+	Nov. 2, 1917, expelled a dead fetus. <i>Bact. abortus</i> isolated therefrom.
Nov. 14, 1917	+	+	+	+	P	P	+	+	+	+	+	
Nov. 28, 1917	+	+	+	+	P	P	+	+	+	+	+	
Dec. 13, 1917	+	+	+	+	P	P	+	+	+	+	+	







TABLE V.—Detailed records of individual experimental animals—Continued  
CONTROL HEIFER NO. 430

Serological results												Remarks
Date	Agglutination					Complement fixation						
	0.04	0.02	0.01	0.005	0.002	0.001	0.04	0.02	0.01	0.005	0.002	
Feb. 28, 1918	—	—	—	—	—	—	—	—	—	—	—	Jab. 7, 1919, bred. Feb. 24, 1919, received 20 cc. fetal-stomach contents (case 80). Mar. 19, 1919, received 25 cc. fetal-stomach contents (case 88).
July 11, 1918	—	—	—	—	—	—	—	—	—	—	—	
Mar. 7, 1919	—	—	—	—	—	—	—	—	—	—	—	
Mar. 21, 1919	—	—	—	—	—	—	—	—	—	—	—	
June 25, 1919	+	+	+	+	+	+	+	+	+	+	+	
July 10, 1919	+	+	+	+	+	+	+	+	+	+	+	Aug. 13, 1919, expelled a dead fetus. <i>Bact. abortus</i> isolated from fetus by cultural methods. Oct. 6, 1919, slaughtered. <i>Bact abortus</i> isolated from udder by cultural methods. Failure experienced in isolation of <i>Bact. abortus</i> from uterus culturally.
July 25, 1919	+	+	+	+	+	+	+	+	+	+	+	
Aug. 23, 1919	+	+	+	+	+	+	+	+	+	+	+	
Sept. 6, 1919	+	+	+	+	+	+	+	+	+	+	+	
Sept. 19, 1919	+	+	+	+	+	+	+	+	+	+	+	

## CONTROL COW NO. 470

Dec. 20, 1918	—	—	—	—	—	—	—	—	—	—	—	Feb. 24, 1919, bred.
Jan. 8, 1919	—	—	—	—	—	—	—	—	—	—	—	
Jan. 22, 1919	—	—	—	—	—	—	—	—	—	—	—	
Feb. 7, 1919	—	—	—	—	—	—	—	—	—	—	—	
Feb. 21, 1919	—	—	—	—	—	—	—	—	—	—	—	
Mar. 7, 1919	—	—	—	—	—	—	—	—	—	—	—	May 24, 1919, received 50 cc. fetal-stomach contents (case 92).
Mar. 21, 1919	—	—	—	—	—	—	—	—	—	—	—	
Apr. 4, 1919	—	—	—	—	—	—	—	—	—	—	—	
Apr. 19, 1919	—	—	—	—	—	—	—	—	—	—	—	
May 3, 1919	—	—	—	—	—	—	—	—	—	—	—	
May 17, 1919	—	—	—	—	—	—	—	—	—	—	—	Oct. 11, 1919, three guinea pigs inoculated with a sample of milk; when destroyed, Nov. 26, 1919, gave negative agglutination reactions and negative cultural results for <i>Bact. abortus</i> .
May 30, 1919	—	—	—	—	—	—	—	—	—	—	—	
June 14, 1919	P	P	—	—	—	—	—	—	—	—	—	
June 25, 1919	P	S	—	—	—	—	—	—	—	—	—	
July 10, 1919	P	S	—	—	—	—	—	—	—	—	—	
July 25, 1919	P	—	—	—	—	—	—	—	—	—	—	Oct. 22, 1919, <i>Bact. abortus</i> isolated from milk by cultural methods and guinea-pig inoculations.
Aug. 9, 1919	P	—	—	—	—	—	—	—	—	—	—	
Aug. 23, 1919	P	S	—	—	—	—	—	—	—	—	—	
Sept. 6, 1919	+	+	P	P	P	—	+	+	+	+	P	
Sept. 19, 1919	+	+	+	+	P	P	+	+	+	+	+	
Oct. 4, 1919	+	+	+	+	P	P	+	+	+	+	+	Nov. 17, 1919, expelled a weak calf. Placenta showed excessive necrosis. Three guinea pigs inoculated with an emulsion of placenta, when destroyed, Jan. 7, 1920, gave positive agglutination reactions and positive cultural results for <i>Bact. abortus</i> .
Oct. 17, 1919	+	+	+	+	P	P	+	+	+	+	+	
Oct. 31, 1919	+	+	+	+	P	P	+	+	+	+	+	
Nov. 14, 1919	+	+	+	+	P	P	+	+	+	+	+	
Nov. 29, 1919	+	+	+	+	P	P	+	+	+	+	+	
Dec. 13, 1919	+	+	+	+	P	P	+	+	+	+	+	
Jan. 3, 1920	+	+	+	+	P	P	+	+	+	+	+	

TABLE V.—Detailed records of individual experimental animals—Continued  
CONTROL COW NO. 453

Serological results												Remarks
Date	Agglutination						Complement fixation					
	0.40	0.02	0.01	0.005	0.002	0.001	0.04	0.02	0.01	0.005	0.002	
Feb. 28, 1920	—	—	—	—	—	—	—	—	—	—	—	Dec. 18, 1919, bred.
Mar. 13, 1920	—	—	—	—	—	—	—	—	—	—	—	
Mar. 27, 1920	—	—	—	—	—	—	—	—	—	—	—	
Apr. 10, 1920	—	—	—	—	—	—	—	—	—	—	—	
Apr. 24, 1920	—	—	—	—	—	—	—	—	—	—	—	
May 7, 1920	—	—	—	—	—	—	—	—	—	—	—	May 10, 1920, received 22 cc. fetal-stomach contents (case 126).
May 21, 1920	—	—	—	—	—	—	—	—	—	—	—	
June 5, 1920	+	P	S	—	—	—	P	—	—	—	—	June 22, 1920, expelled a dead fetus. Placenta necrotic. <i>Bact. abortus</i> isolated from placenta by cultural methods and guinea-pig inoculations.
June 18, 1920	+	+	+	+	P	S	+	P	—	—	—	
July 2, 1920	+	+	+	+	P	P	+	+	+	P	—	July 2, 1920, three guinea pigs inoculated with milk; when destroyed, Sept. 3, 1920, gave positive agglutination reactions and positive cultural results for <i>Bact. abortus</i> .
July 18, 1920	P	+	+	P	P	P	+	+	+	+	—	
July 30, 1920	P	+	+	+	P	P	+	+	+	+	P	
Aug. 13, 1920	P	+	+	+	+	P	+	+	+	+	S	
Aug. 27, 1920	P	+	+	+	+	P	+	+	+	+	+	
Sept. 10, 1920	P	+	+	+	+	P	+	+	+	+	+	
Sept. 21, 1920	P	+	+	+	+	P	+	+	+	+	+	

# THE RHIZOCTONIA BROWN ROT AND OTHER FRUIT ROTS OF STRAWBERRIES<sup>1</sup>

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## INTRODUCTION

The fruit rots of strawberries have received much less attention than the importance of the crop and the great losses from decay would seem to justify. A number of fungi are known which attack strawberries and it is to be expected that as investigation continues there will be discovered numerous other species which in certain localities or under some conditions are capable of causing serious fruit rots. Very recently Rose<sup>2</sup> has published the results of an extensive study of a fruit rot of strawberries found to be destructive in the Arkansas and Tennessee regions and which he calls "leather rot." In the present paper the writers report a new or little known field rot which is very important in Florida. Since both these diseases produce a brown discoloration, and at least two other so-called "brown rots" of strawberries are already known, it seems necessary to briefly discuss at this time the distinguishing characters of these rots.

## THE BROWN RHIZOCTONIA ROT

Each season since 1920 as strawberries have come into the Washington market from Florida the writers have observed a peculiar rot on many of the berries which appeared to be perfectly sound on one side. Some of these berries were deformed as though one side had failed to fill out properly. This part of the berry was commonly affected with a somewhat dry brown rot which often had a blackish color, due chiefly to the adhering black sandy soil. This suggested that the rot might be due to some soil organism.

Study of the rots in Florida strawberry fields, especially in the vicinity of Plant City in 1923, and Kissimmee in 1924, shows that while *Botrytis* appears in epidemics, suddenly and with very destructive effects during or soon after brief periods of rainy weather, the rot just mentioned as found in the Washington markets develops much more regularly in the fields and makes it necessary to cull out large numbers of berries during picking and packing. As this disease is almost always present, yet rarely if ever epidemic, it attracts relatively little attention among growers. Continued observation has convinced the writers, however, that it is a constant source of loss to strawberry growers in central Florida and, until a remedy is discovered, will remain a handicap to the industry. Careful observation has convinced the writers that *Rhizoctonia* caused at least half the loss from field rots in this region during the season of 1923-24.

The characters of this rot are so pronounced that it can be distinguished readily from the other so-called brown rots of strawberry. Affected berries are generally one-sided, and show a hard brown rot, to which often adhere quantities of sandy soil. Decay regularly starts on the under side of the berry where it comes in contact with the soil, and a small amount of soil will usually be found clinging to the decayed spot even in dry weather. If the soil is of the black

<sup>1</sup> Received for publication Feb. 25, 1924

<sup>2</sup> ROSE, D. H. LEATHER ROT OF STRAWBERRIES. Jour. Agr. Research, 28: 357-376, illus. 1924.

sandy type common in low places in many Florida strawberry fields, the surface of the rotting area will of course appear blackish (Pl. 1, A, C). If little or no soil clings to the berry, the true dark brown color of the rot will be evident (Pl. 1, D). The rot usually starts before the berry begins to turn red, sometimes even before the fruit is a third grown (Pl. 1, E). In this case the discolored side will be of a lighter brown, and only a little soil will adhere. An early attack on the berry will result in a deformed fruit, but as the rot progresses rather slowly, the upper side of the berry develops normally and ripens without showing rot, so that one can not tell that the berry is diseased until it is turned over. As a result many such berries are picked unintentionally and only extreme care will prevent their being overlooked by packers.

Sections of the rotting berry show that there is a definite line of demarcation in the pulp indicating clearly how far the disease has spread. Where healthy and diseased tissues meet, the pulp is only slightly faded and brownish; otherwise the diseased area stands out sharply on account of its rich brown color. The rotted part later becomes dried out or mummified (Pl. 1, B). By using a hand lens one is able to see masses of hyphae which bind the particles of soil together and hold the dirt to the surface of the berry. It will be shown that these soil hyphae belong to the *Rhizoctonia* which is causing the rot. In a free-hand section of the browning pulp examined under the microscope can be seen vast numbers of hyphae crowding in between the cells in the more recently invaded regions (Pl. 2, B, a), while in those parts of the berry where the fungus has completed its work the cells are crushed and distorted and the spaces are packed with felty masses of mycelium. If the affected part of the berry is cut away the remainder will be found to be perfectly edible (Pl. 1, C). Such rot fungi as *Botrytis* and *Rhizopus* grow through the berry and usually completely destroy it once they have gained a foothold. The appearance of berries attacked by the hard rot is such that they will be discarded wherever found. The rot develops so slowly that it would not be communicated rapidly to other berries under refrigeration in transit.

Whether the fungus, as it advances between the cells, attacks only the middle lamellae, as is so frequently reported for other intercellular fungi, is uncertain. At first the cells of the invaded tissues actually become hypertrophied, consequently the intercellular hyphae are put under pressure and are compelled to force back the elastic cell walls as they advance (Pl. 2, B, c). Certainly water is not withdrawn from the pulp cells in mass, resulting either in a wet rot such as is produced by *Rhizopus* or in a separation or falling apart of host cells, such as follows the work of *Pezizella*. Water seems to be lost more by a drying out process. Cells in the decaying tissues gradually collapse after the period of swelling and the hyphae crowd in, becoming more and more intracellular. The feeding of the hyphae in the middle lamellae may be the cause of a sort of mucilaginous disorganization which is manifested by the presence of quantities of intercellular stainable substance which so often surrounds the hyphae. This is more noticeable as the disease progresses. Striae or laminations in the adjacent walls are then more distinct. The intercellular substance and the peculiar manner in which the mucilaginous layers are split apart are brought out in Plate 2, figure B, b. The contents of the unstained hyphae are foamy or vacuolate.

### MYCELIAL CHARACTERS

The mycelium of the fungus is comprised of septate branching hyphae of fairly uniform diameter, though where a branch grows out the diameter is somewhat less. In some cases hyphae are very coarse for short distances and branches are very irregular with blunt ends. The cells usually contain more than one

nucleus, sometimes as many as five; two or three being the common numbers. The nuclear wall is not very distinct in stained preparations but the large nucleole is always plainly visible, surrounded by a clear place in the cytoplasm (Pl. 2, A, b).

### ARTIFICIAL INOCULATIONS ON STRAWBERRIES

Strawberries growing in pots in the greenhouse at the Arlington experiment farm, Virginia, were readily inoculated by laying pieces of agar bearing mycelium on the soil beneath half-grown berries. In every case the berries developed the characteristic rot within three or four days. The fungus penetrated the soil at the point attacked so that when the berry was removed some of the soil was attached to it. The fungus was recovered from such diseased berries by transfer of pulp from points well below the surface. Greenish berries from the Washington market have also been inoculated in damp chambers by placing bits of agar covered with hyphae from test-tube cultures on the bottom of damp chambers and then laying an apparently sound berry on the piece of agar. The brown rot developed in every case within two days (Pl. 1, G). Since the epidermis of a strawberry is so liable to rupture from all sorts of causes it is not certain that the fungus actually penetrated the unbroken epidermis.

Sections of berries artificially inoculated show that the hyphae crowd in between the akene and that part of the receptacle in which the akene is partly sunken. It may be that the fungus finds a film of moisture in this region where the walls of the epidermis are also easily penetrated. The most noticeable effect upon the host tissue is a swelling and clearing of the cells between which the hyphae are pushing. The cell contents take very little stain. The fungus apparently does not seek out any particular part of the berry. Hyphae are found in every tissue in the region attacked. The sieve tubes or long conducting cells of the vascular bundles leading to the "seeds" are pushed aside and hyphae crowd in between them or run along between these cells in all directions (Pl. 3, A). Hyphae also pack in between the small cells of the pulp between the akenes and crowd up close to the cells of the sclerotized and thickened cell walls (Pl. 3, B). No living tissue appears to be immune. If nuclei were present in the diseased pulp cells examined, they did not take the stain. The nuclei of the fungus, however, appeared very distinctly (Pl. 2, A, b; B).

### THE TAN BROWN ROT OF STRAWBERRIES

An excellent description of the *Pezizella* brown rot of strawberries was published by Stevens and Peterson<sup>3</sup> in connection with their account of the organism causing the disease. They described this fungus as *Patellina fragariae* although it had already been described under a great many different names.<sup>4</sup> The region attacked by the fungus is marked on green berries by the appearance of small sunken spots. These spots are tan colored, slightly sunken, and enlarge only slowly. When a ripe berry is attacked, the rot spreads much more rapidly but the color of the spot is about the same (Pl. 1, H). The fungus grows into the pulp of the berry so that the rotted portion is deeper than its diameter. The core of the region attacked is consumed and the host tissue is replaced by mycelium so that the decayed portion finally presents a spongy dry texture. The host tissue immediately surrounding the diseased area, although devoid of mycelium, becomes soft and wet and its cells separate from each other. As the core of the rotten spot is very tenacious it can easily be removed intact. This

<sup>3</sup> STEVENS, F. L., and PETERSON, A. SOME NEW STRAWBERRY FUNGI. *Phytopathology*. 6: 258-267, illus. 1916.

<sup>4</sup> SHEAR, C. L., and DODGE, B. O. THE LIFE HISTORY AND IDENTITY OF "*PATELLINA FRAGARIAE*," "*LEPTOTHYRIUM MACROTHECIUM*" AND "*PEZIZA OENOTHERAE*," *Mycologia*, 13: 135-170. pl. 8-10. 1921.

characteristic serves for the ready identification of this rot. In addition it is often possible to see with the aid of a hand lens the little "sporodochia" of the *Hainesia* (*Patellina*) stage of the fungus on the surface of the spot. Teased and microtome sections show, according to Stevens and Peterson, that the host cells are filled with hyphae.

### THE LEATHER ROT OF STRAWBERRIES

The leather rot, as Rose<sup>5</sup> calls the disease, is caused by a *Phytophthora* very similar to *P. cactorum* and is characterized by a definite though slight softening of the affected tissues and by internal discoloration. It is a typical field rot attacking berries in all stages of growth, and is most common following rainy periods, a characteristic which results in the name "water soak" often used by growers. The results of Rose's inoculation experiments did not lead him to conclude that the hyphae of this *Phytophthora* can penetrate the unbroken strawberry epidermis. In the field, however, berries touching the ground were first affected on the under side, suggesting that the fungus may be a true soil organism, and that it may be able to penetrate the unbroken epidermis under favorable circumstances.

Green fruit becomes brown where attacked, and fruit that has turned red before the fungus gains entrance presents a series of color changes as the disease progresses. At the center of the spot the color becomes yellow to light brown, spreading out from here the color changes to purple and then to the natural red as sound tissue is approached (Pl. 1, J). The most characteristic feature of the rot is shown in sections of the fruit. The vascular tissue becomes markedly discolored, being a much deeper brown than the affected pulp (Pl. 1, K). In the early stages of the disease vascular browning may be the only symptom. There is no clear line of demarcation between sound and diseased tissue, such as is characteristic of the hard brown rot. Neither can the diseased tissue be scooped out as it can be in the case of the *Pezizella* rot. One striking characteristic of leather rot is that even slightly rotten berries are bitter to the taste.

### THE BOTRYTIS BROWN ROT OF STRAWBERRIES

Strawberries attacked by *Botrytis* present symptoms which in the early stages of the disease are quite different from those shown in the later stages. As the hyphae penetrate into the pulp it loses its natural color and turns light brown. Later the color becomes somewhat darker (Pl. 1, I). The rotting pulp is at first rather soft, almost watery, though never leaky. This stage is soon superseded by a drying out so that the berry becomes firm. After the disease has run its course, the berry is found to be hard and dry,<sup>6</sup> which results in the use of the term "dry rot" among growers. The tendency, however, to make use of color characters describing rots will always prevail to a certain extent, so that shippers and inspectors no doubt will continue to use the term brown rot for this and similar diseases.

As soon as aerial hyphae with their crop of conidia develop, the surface of the berry presents a characteristic appearance which is referred to as "gray mold." Sections of immature or green berries recently affected with *Botrytis* rot do not show a distinct line of demarcation between healthy and diseased tissue. The diseased pulp is of a darker brown near the surface. The color shades off to a lighter brown and ends with the natural color of the healthy fruit in parts not invaded by the mycelium. Stevens<sup>7</sup> who investigated the host parasite relation

<sup>5</sup> ROSE, D. H. LEATHER ROT OF STRAWBERRIES. Jour. Agr. Research, 28: 1924.

<sup>6</sup> STEVENS, NEIL E., and WILCOX, R. B. FURTHER STUDIES OF THE ROTS OF STRAWBERRY FRUITS. U. S. Dept. Agr. Bul. 686, 14 p. 1918. Literature cited, p. 14.

<sup>7</sup> STEVENS, NEIL E. PATHOLOGICAL HISTOLOGY OF STRAWBERRIES AFFECTED BY SPECIES OF BOTRYTIS AND RHIZOPUS. Jour. Agr. Research 6: 361-366, pl. 49-50. 1916.

in connection with this disease found that the hyphae penetrate the cell walls readily and dissolve the middle lamellae. The hyphae were found to grow between the cells for some distance, then to penetrate the cell walls becoming intracellular. All parts of the berry are subject to attack by the fungus, which if allowed to develop does not stop at localized infections. It is evident from the way the cytoplasm shrinks away from the cell walls and becomes disorganized that the host cells are killed quickly. Water is so slowly and gradually withdrawn from the host cells that it evaporates from the surface of the berry and no leak is caused, in spite of the fact that hyphae penetrate pulp or storage cells everywhere. The rotting berry thus becomes dried and mummified.

#### COMPARISON OF THE FOUR BROWN ROTS

The two field rots most likely to be confused with the hard brown rot are those caused by *Botrytis* and *Pezizella*, although each has sufficiently distinctive characters to make identification reasonably certain. The *Botrytis* rot is a lighter brown and more watery at the beginning. That caused by *Pezizella* forms a pocket in the sticky scablike rotted portion. In only one case out of 126 cultures made by direct transfer of pulp from berries selected in the field as affected with hard brown rot was *Botrytis* obtained as a result of faulty diagnosis. The hard rot caused by the *Rhizoctonia* under discussion in this paper is easily distinguished from the tough dry rot due to *Botrytis* by the clear line of demarcation between healthy and diseased tissue characteristic of the *Rhizoctonia* rot.

The *Rhizoctonia* hard brown rot and the *Pezizella* soft brown rot are alike in that in both there is a distinct line separating the diseased from the healthy tissue. Not infrequently berries infected with the *Rhizoctonia* when shipped arrive at destination with the uninfected portion of the berry still edible. A berry infected with *Botrytis* or *Phytophthora* when shipped is usually so rotten as to be wholly inedible on arrival. The leather rot (*Phytophthora* rot) is the only one of the four brown rots to cause a marked vascular discoloration.

The surface of berries affected with *Phytophthora* under moist conditions becomes covered with a pure white growth of hyphae and conidiophores (Pl. 1, J). Those affected with *Botrytis* develop the gray mold which consists in part of branched conidiophores. The *Pezizella* rot develops little disc-shaped fruiting bodies sometimes called sporodochia. The *Rhizoctonia* hard rot develops only light brown, fluffy hyphae.

The mycelium of *Botrytis* penetrates the host cells in every direction and is both inter- and intracellular. The hyphae of the hard brown rot fungus are typically intercellular, but they are often found within the cells. Hyphae of the *Pezizella* are probably capable of penetrating the walls of the pulp cells but are not known to penetrate the cutinized epidermis. Little is known as to the manner in which the hyphae of the leather rot attack the host cell.

#### KNOWN DISTRIBUTION OF THE VARIOUS BROWN ROTS

The *Rhizoctonia* rot is now known from central Florida, North Carolina and possibly from Tennessee. Leather rot has been found in Mississippi, Louisiana, Arkansas, Tennessee, Missouri, Kentucky, and Illinois. *Pezizella* rot has been observed by the writers in Cuba, Louisiana, Florida, Arkansas, Virginia, Maryland, Wisconsin, and Alaska. *Botrytis* has been found to some extent as a fruit rot of strawberries in every strawberry region visited, though it varies greatly in abundance.<sup>8</sup> It is serious in Alaska<sup>9</sup> and during many seasons in New England, and may become abundant in more southern regions during wet weather. *Botrytis* is also known to cause rot of strawberries in England.

<sup>8</sup> STEVENS, NEIL E. ROTS OF EARLY STRAWBERRIES IN FLORIDA AND SOUTHERN CALIFORNIA. Amer. Jour. Bot., 9: 204-211. 1922. Literature cited, p. 211.

<sup>9</sup> ANDERSON, J. P., BOTRYTIS CINEREA IN ALASKA. Phytopathology. 14: 152-155. 1924.



## CONTROL

Investigations as to the possibility of the control of the various field rots of strawberries by spraying and dusting are now in progress. The results which have apparently been obtained by a few growers indicate the possibility of success but it will be several years before definite recommendations can be made. Covering the ground with a mulching of pine needles is occasionally practiced in Florida, and apparently results in a lessening of the rot caused by the *Rhizoctonia*.

## SUMMARY

A fruit rot of strawberries found to be important in central Florida is caused by a fungus which in its mycelial characters resembles *Rhizoctonia solani* Kühn as commonly found on cultivated plants.

When the berry is first attacked by this fungus the cells of the invaded tissues become somewhat hypertrophied. Later the cells of the decaying tissues gradually collapse and the mycelium of the fungus which is at first intercellular becomes both inter and intracellular.

The "hard brown rot" caused by this *Rhizoctonia* is characterized by the fact that it regularly starts on the under side of the berry, advances slowly and shows a definite line of demarcation between the brown decayed portion and the normal uninfected pulp. In addition the berry is often distorted or "one-sided" in shape and particles of soil usually adhere tightly to the affected area.

The "tan rot" caused by *Pezizella lythri* is confined to a somewhat cone-shaped region, the base of which appears as a tan-colored, slightly sunken spot on the side of the berry. This rotted portion may readily be separated almost intact from the sound tissue.

The leather rot, caused by a species of *Phytophthora* is easily distinguished, if the fruit is cut open, by the fact that the vascular tissue becomes markedly discolored being a much deeper brown than the affected pulp. Berries rotted by this fungus are very bitter to the taste.

The *Botrytis* or grey mold rot is not localized in any part of the berry as the fungus grows readily in all tissues. Berries affected by *Botrytis* are brown and rather tough without either the marked vascular discoloration or localized decay which are characteristic of one or more of the other brown rots discussed in this paper.

## PLATE 1

A.—Missionary strawberry affected with the *Rhizoctonia* hard brown rot. The blackish discoloration of the berry is due in part to the color of the sandy soil adhering. The particles of soil are held together and bound to the berry by the mycelium of the fungus. The brown discoloration due to the rot shows more clearly next to the calyx.

B.—Cross section of the berry shown in A. Here the very dark brown band above consists of a rather hard felty mass made up of mycelium and host cells overlaid with black soil. The lighter brown layer of pulp shows to what point the fungus has penetrated.

C.—Another Missionary strawberry characteristically marked as a result of infection by the *Rhizoctonia*.

D.—Early stages of infection of berry with the hard brown rot. The true color of the rot is not masked by adhering soil in this case.

E.—Green berry one-third grown attacked where it lay in contact with the soil.

F.—Diseased immature berry from the Washington markets after shipment by express from Florida under refrigeration. Pure cultures of the *Rhizoctonia* were obtained from this berry by a transfer of diseased pulp.

G.—Section of berry two days after inoculation with the *Rhizoctonia* by laying a piece of agar bearing mycelium on the surface of the berry.

H.—Strawberry showing the tan brown rot caused by *Pezizella*. Note the sunken area.

I.—Green berry a short time after infection by *Botrytis*. Note the brown discoloration of the fruit beneath the gray mould.

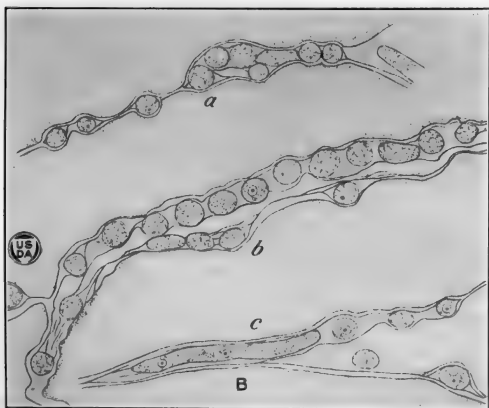
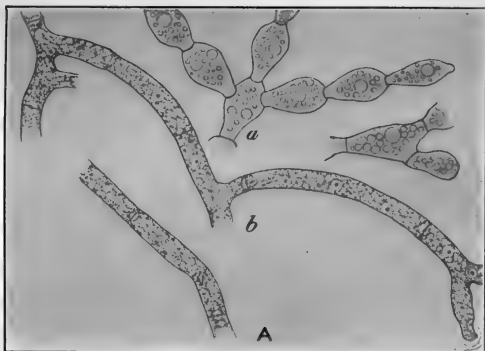
J.—Aroma strawberry from Arkansas affected with the *Phytophthora* Leather Rot.

K.—Section of the same kind of berry showing vascular browning.

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Figures A to I from colored drawings by J. Marion Shull; figures J and K, from colored photographs contributed by Dr. D. H. Rose.





## PLATE 2

Mycelium of the *Rhizoctonia* from a sclerotium and from the large pulp cells of Missionary strawberry, drawn by the aid of the camera lucida A. Zeiss 4 mm. 8 oc.; B, C. 1.5 mm. Obj., 8 oc. Reduced about one-third.

A.—(a) Terminal branches from a sclerotium, unstained. Most of the cells in this case contain one or two large oil globules and several smaller ones. (b) Intracellular hyphae showing character of branching, crescent-shaped hyaline areas at the cross walls and two or three nuclei in each cell. Stained with Flemming's triple stain.

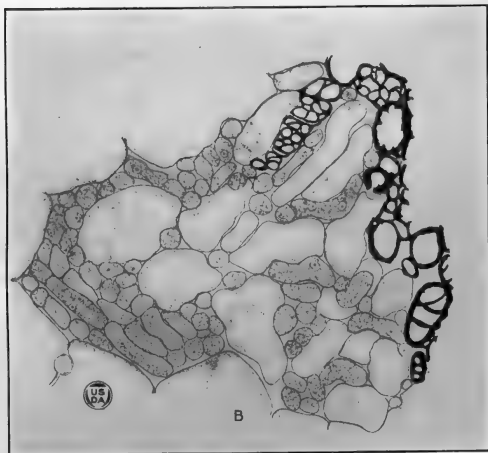
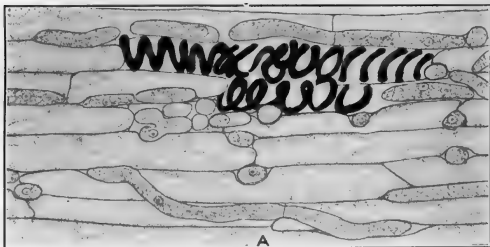
B.—(a) Intercellular hyphae in cross section pushing back the elastic walls of the turgid pulp cells. Note the intercellular substances, possibly derived from disorganized middle lamellae; (b) similar to the preceding except that the intercellular substances appear to be in more or less distinct layers. Cytoplasmic contents of pulp cells indicated by stippling. Large vacuoles filled with cell sap. Intercellular substance or disorganizing laminae of the walls shown by wash drawing; (c) hyphae pushing forward between two cells which at the right have spread apart to form an open intercellular space.

### PLATE 3

Mycelium of the *Rhizoctonia* in the tissues of Missionary strawberry. Drawn with the aid of a camera lucida, Zeiss, 1.5 mm. Obj., 8 oc. Reduction about one-third.

A.—Section along the edge of a vascular bundle in the pulp. Hyphae push in between the long conducting cells forcing their walls aside. No intercellular spaces are present owing to the turgescence of the affected cells. Hyphae penetrate the vascular tissues in all directions.

B.—Section just beneath an akene, showing how the very small pulp cells in this region are entirely surrounded by intercellular hyphae which push up closely to the fruit or seed coats whose cells are thickened or sclerotized. No nuclei are visible in the host cells.



# EFFECTIVE USE OF HYDROCYANIC-ACID GAS IN THE PROTECTION OF CHICK-PEAS (*CICER ARIETINUM*) WAREHOUSED IN 240-POUND SACKS <sup>1</sup>

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One of the desiderata connected with the protection from insect attack of food commodities in storage is complete information regarding the penetration of fumigants. Hydrocyanic-acid gas is recognized as an effective and standard fumigant for the control of many pests under a wide range of conditions, but always in situations where the gas can be confined sufficiently long to reach and kill the insects. This fumigant as ordinarily used is most effective in the treatment of containers that are reasonably empty; in other words, in fumigation work which requires no great penetration of the gas. It is used with excellent results in the fumigation of houses, hospitals, railway passenger and sleeping cars, certain freight cars, ships from which cargoes have been removed, flour mills and similar structures. It is generally conceded that in the protection of commodities stored in bulk, such as wheat and corn in farmers' bins, grain cars or commercial elevators, or stored in sacks and stacked tightly in warehouses, as in the case of many animal feeds, rice, and other seeds, hydrocyanic-acid gas is far less effective and can not be depended upon for sufficient penetration to rid the commodity of its living insect inhabitants.

The use, however, of hydrocyanic-acid gas is much to be desired over that of carbon disulphid, which is a standard fumigant for such commodities, inasmuch as it can be used in large bulks in congested city districts where the generation of an equally large volume of the inflammable and explosive carbon disulphid gas would be quite out of question because of the fire hazard. For these reasons data bearing upon the power of penetration of hydrocyanic-acid gas into the usual warehoused commodities habitually affected by insects have a most practical application in conservation work. Food commodities in storage are of great value, under private ownership, subject to removal on short notice from storage and to shipment according to trade conditions, and therefore are not often available for prolonged scientific study. These are facts which enhance the rare opportunities that are offered Federal investigators to study privately owned stored products.

An unusual opportunity was presented the writers during the years 1918-1920 to observe the disastrous attack of the four-spotted bean weevil *Bruchus quadrimaculatus*, the lesser grain borer *Rhizopertha dominica*, the rice weevil *Sitophilus oryza*, the Angoumois grain moth *Sitotroga cerealella*, the Indian meal moth *Plodia interpunctella*, and the tobacco beetle *Lasioderma serricornis*, upon a large supply of chick-peas, *Cicer arietinum*. These edible legumes, amounting to about 137,000 sacks of 240 pounds each, represented for the most part the greater portion of the crop of this food grown in northern Mexico during 1918 and diverted from storage in New York City by congested war conditions to six warehouses in New Orleans. This crop had arrived at New Orleans by rail during the summer of 1918 and had been stored under both favorable and unfavorable conditions in space permitted in lots of 15,000, 12,000, 30,000, 30,000, 30,000, and 20,000 sacks, respectively, in the six warehouses. During

<sup>1</sup> Received for publication April 22, 1924.



early October, 1918, the owners discovered that while the bulk of the consignments was uninfested, many carload lots of the seeds showed heavy infestation and were heating badly as a result of bruchid attack. The centers of infestation were, unfortunately, so distributed throughout the warehouses that rapid spread in infestation was taking place, and the heating caused by the infestations was offsetting the normal checks of colder winter weather upon weevil activity and spread. Numerous sacks of seeds had already been rendered unfit for food both by the bruchids and the *Rhizopertha*. Since the seeds had a retail value of about \$5,000,000, action was imperative. As the warehouses, with one exception, were within the congested business districts of the city only a noninflammable gas could be used. Hydrocyanic-acid gas was chosen by the owners, who engaged a professional fumigator. This operator fumigated the warehouses, using  $2\frac{1}{2}$  pounds of sodium cyanid 98 to 99 per cent pure for each 1,000 cubic feet of space. The senior writer was present at the time of the fumigations and took numerous samples, the results of the examinations of which are only in part reported upon in this paper. The fumigations were allowed to extend over a period of 48 hours, although the warehouses were not as a rule tight enough to confine the gas that long. The writers were not permitted to experiment with the dosage chosen by the owners. While the cost of using so large a dosage greatly increased the cost of fumigation above what was necessary in the opinion of the writers, the cost was nothing in the eyes of the owners when compared to the value of the commodity. The fumigations were conducted when the average mean temperatures ranged from 58° to 75° F.

In taking samples both before and after fumigations, the writers used the usual grain car probe, about 5 feet long, as illustrated (Pl. 1, C). This probe was thrust from end to end of the sack through the central axis and the samples (usually six) placed separately in sacks and numbered according to their relative positions in the bulk of the sack sampled. In making the intensive examination later only 50 of the seeds of a sample were used, the remainder being held for future observation.

Diagrams are presented hereafter showing the location of the sacks from which samples were taken after fumigation, together with data on the degree of infestation and penetration of the gas and the effect of the fumigant upon the insects. It happens that the data on infestations serve to indicate also both the penetration and the effectiveness of the fumigant, for the reason that in no sample recorded in the following pages were any insects found alive, and no development occurred subsequently from eggs on the seeds or from larvæ or pupæ within the seeds at the time of fumigation, as shown by examination made at intervals during two years following the fumigations. No extended examinations were made until about one month after the fumigations, when the dead larvæ and pupæ had had time to discolor if killed. All seeds examined for larvæ and pupæ were first softened and then sliced with scalpels to reveal infestation. The references to the degree of larval development, such as one-eighth, one-fourth, one-half, or full-grown, are of course approximate statements based upon the general appearance of the individual.

#### WAREHOUSE NO. 1

The 30,000 sacks of chick-peas stored in this warehouse were in a room on the third (top) floor, measuring 150 by 150 by 20 feet. The warehouse was of brick construction, with a tar and pebble roof. Leakage of gas about the roof during fumigation was rather great, little or no discomfort being experienced when the room was opened for ventilation 48 hours after fumigation was started. The fumigator used 80 fifty-gallon oil barrels, each generating a charge containing 15 pounds of sodium cyanid. The capacity of this warehouse was greatly overtaxed (Pl. 1, A), the sacks being piled from 6 to 17 deep, whereas the usual

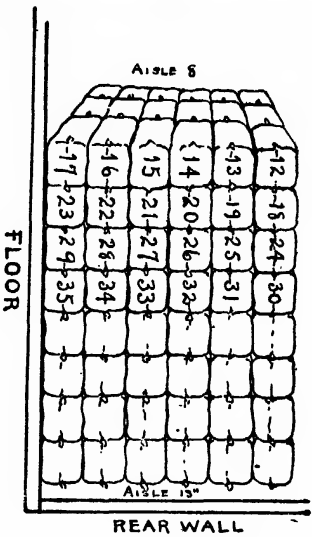
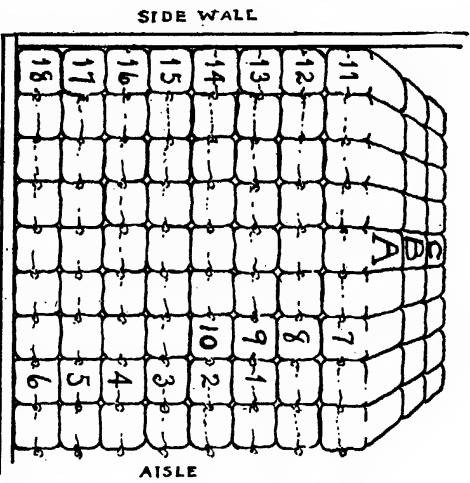
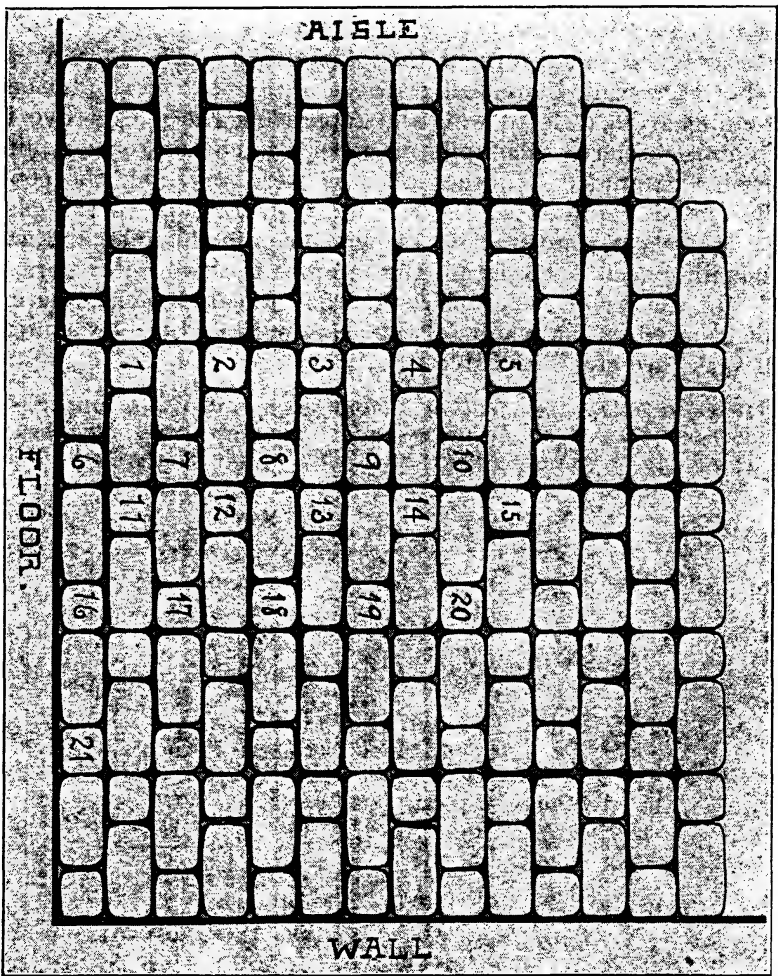


FIG. 1.—Arrangement of sacks in (a) Warehouse No. 1, (b) Warehouse No. 2, and (c) Warehouse No. 3, from which samples were taken and reported on in Tables I to III, respectively.

custom for storing this commodity is to pile the sacks but 5 or 6 deep. The floor was of wood. Coffee was stored on the floor below.

After fumigation, samples were taken from sacks 1 to 21, located in a pile of sacks, as indicated in figure 1, *a*. This pile of sacks consisted of five tiers, of which the sampled sacks formed the central tier. The lot was piled directly against the brick wall, there being spaces of about 2 feet on either side of the pile, extending from aisle to wall and separating the pile of five tiers of sacks from neighboring tiers. This pile of sacks was chosen for sampling because it was as well surrounded by other piles as were any, and the central tier was chosen because it represented the tier most inaccessible to the gas. It will be noted that the sacks were stacked 14 sacks deep in this pile, and an interlocking system of piling used. To obtain the samples the two outer tiers of sacks were removed and the probe thrust through the indicated sacks, the ends of which were thus exposed. The results of the examination of the six samples from each of the 21 sacks are given in Table I.

TABLE I.—Data on the penetration of hydrocyanic-acid gas into 240-pound sacks of chick-peas infested by *Bruchus quadrimaculatus* and arranged as shown in figure 1, *a*. All bruchids reached and killed by the fumigant

Sack No.	Sample No.	Number of seeds examined	Number of seeds infested	Number of—								Total larvæ, pupæ, and adults	Eggs unaccounted for
				Eggs	Emergence holes	Larvæ				Pupæ	Adults		
						One-eighth	One-fourth	One-half	Full-grown				
1-----	1	50	47	321	14	39	19	17	70	17	19	181	126
	2	50	45	167	1	31	8	16	30	3	10	98	156
	3	50	12	43	0								43
	4	50	26	19	3	1	1	0	5	3	8	18	
	5	50	26	57	3	13	2	4	4	2	3	28	26
	6	50	21	150	2	13	3	4	5	2	0	27	121
		300	177	757	23	97	33	41	114	27	40	352	472
2-----	1	50	48	140	19	2	6	10	36	5	46	105	16
	2	50	18	43	1	5	4	6	9	1	8	33	9
	3	50	9	22	6	3	0	1	1	2	2	9	7
	4	50	15	20	3	3	3	0	0	0	1	7	10
	5	50	22	27	1	5	4	1	8	1	2	21	5
	6	50	40	117	3	42	22	0	9	3	0	76	38
		300	152	369	33	60	39	18	63	12	59	251	85
3-----	1	50	43	185	18	13	5	8	21	15	25	87	80
	2	50	14	20	1	1	0	1	1	3	2	8	12
	3	50	7	13	1	1	0	1	1	1	3	7	5
	4	50	8	12	1	2	0	1	2	1	3	9	2
	5	50	10	15	4	0	1	2	3	1	5	12	
	6	50	19	26	0	6	4	3	0	3	0	16	10
		300	101	271	25	23	10	16	28	24	38	139	109
4-----	1	50	38	60	3	2	8	8	15	6	0	39	18
	2	50	26	38	5	3	0	5	3	7	1	19	14
	3	50	12	14	1	6	0	0	0	2	0	8	5
	4	50	14	20	0	4	1	2	2	3	0	12	8
	5	50	8	8	0	3	1	1	3	2	0	10	
	6	50	14	17	0	3	5	1	3	5	0	17	0
		300	112	157	9	21	15	17	26	25	1	105	45
5-----	1	50	47	238	42	6	5	9	14	27	69	130	66
	2	50	10	9	5	3	0	0	1	0	4	8	
	3	50	9	9	0	1	0	2	0	1	1	5	4
	4	50	7	4	11	2	1	2	4	0	0	9	
	5	50	10	5	10	2	2	3	2	1	0	10	
	6	50	12	6	20	3	2	3	6	1	2	17	
		300	95	271	88	17	10	19	27	30	76	179	70

TABLE I.—Data on the penetration of hydrocyanic-acid gas into 240-pound sacks of chick-peas infested by *Bruchus quadrimaculatus* and arranged as shown in figure 1, a. All bruchids reached and killed by the fumigant—Continued

Sack No.	Sample No.	Number of seeds examined	Number of seeds infested	Number of—								Total larvae, pupæ, and adults	Eggs unaccounted for
				Eggs	Emergence holes	Larvæ				Pupæ	Adults		
						One-eighth	One-fourth	One-half	Full-grown				
6-----	1	50	30	91	1	12	11	7	6	6	4	46	44
	2	50	22	42	2	10	2	8	1	3	3	27	13
	3	50	19	22	3	3	0	6	4	1	0	14	5
	4	50	11	13	0	3	2	0	2	0	0	7	6
	5	50	28	56	0	1	0	3	0	0	0	4	52
	6	50	38	134	0	13	9	1	4	1	0	28	106
		300	148	358	6	42	24	25	17	11	7	126	226
7-----	1	50	46	230	32	7	4	2	13	12	18	56	142
	2	50	34	71	9	5	3	3	6	8	5	30	32
	3	50	16	19	0	1	1	1	4	3	0	10	9
	4	50	24	37	0	12	4	1	5	2	1	25	12
	5	50	31	49	0	27	1	2	6	2	1	39	10
	6	50	29	55	1	18	0	1	6	0	0	25	29
		300	180	461	42	70	13	10	40	27	25	185	234
8-----	1	50	41	173	12	10	8	17	33	24	9	101	60
	2	50	22	31	3	3	2	3	5	6	3	22	6
	3	50	8	10	0	3	2	0	2	1	0	8	2
	4	50	2	2	0	1	0	0	1	0	0	2	0
	5	50	2	2	0	0	0	0	0	1	0	1	1
	6	50	3	3	0	2	0	0	0	1	0	3	0
		300	78	221	15	19	12	20	41	33	12	137	69
9-----	1	50	0	0	0	0	0	0	0	0	0	0	0
	2	50	0	0	0	0	0	0	0	0	0	0	0
	3	50	1	1	0	0	0	0	0	0	0	0	1
	4	50	7	7	1	0	0	0	0	0	0	0	6
	5	50	16	32	0	8	9	6	3	1	0	27	5
	6	50	44	108	0	22	16	11	18	15	5	87	21
		300	68	148	1	30	25	17	21	16	5	114	33
10-----	1	50	44	180	3	19	4	7	17	7	4	58	119
	2	50	33	83	0	19	4	13	4	5	2	47	36
	3	50	16	24	1	5	3	1	2	0	2	13	10
	4	50	17	19	0	4	0	1	0	2	1	8	11
	5	50	25	37	0	5	10	1	3	2	2	23	14
	6	50	35	63	0	14	4	6	2	7	0	33	30
		300	170	406	4	66	25	29	28	23	11	182	220
11-----	1	50	35	77	0	30	0	7	4	1	0	42	35
	2	50	13	26	0	7	0	0	3	3	0	13	13
	3	50	18	27	0	13	0	3	0	3	0	19	8
	4	50	37	65	0	14	2	10	4	3	0	33	32
	5	50	38	75	1	29	0	14	6	4	1	54	20
	6	50	36	64	2	32	3	5	3	3	4	50	12
		300	177	334	3	125	5	39	20	17	5	211	120
12-----	1	50	41	114	21	5	2	1	7	17	16	48	45
	2	50	25	35	5	5	1	0	1	2	6	15	15
	3	50	7	7	1	0	0	0	3	1	3	7	-----
	4	50	19	30	1	1	2	2	5	3	1	14	15
	5	50	19	24	0	8	3	1	0	7	2	21	3
	6	50	12	14	0	1	3	2	2	2	0	10	4
		300	123	224	28	20	11	6	18	32	28	115	82
13-----	1	50	29	74	2	11	7	7	12	10	2	49	23
	2	50	2	8	5	2	0	0	1	0	3	6	-----
	3	50	3	3	0	1	0	1	2	0	0	4	-----
	4	50	4	4	3	0	1	0	1	0	0	2	-----
	5	50	3	9	4	0	0	2	4	1	0	7	-----
	6	50	11	17	1	7	2	1	1	0	1	12	4
		300	52	115	15	21	10	11	21	11	6	80	27

TABLE I.—Data on the penetration of hydrocyanic-acid gas into 240-pound sacks of chick-peas infested by *Bruchus quadrimaculatus* and arranged as shown in figure 1, a. All bruchids reached and killed by the fumigant—Continued

Sack No.	Sample No.	Number of seeds examined	Number of seeds infested	Number of—								Total larvæ, pupæ, and adults	Eggs unaccounted for
				Eggs	Emergence holes	Larvæ				Pupæ	Adults		
						One-eighth	One-fourth	One-half	Full-grown				
14-----	1	50	27	67	12	15	13	0	0	0	14	42	13
	2	50	12	14	0	2	0	0	0	0	0	2	12
	3	50	17	18	2	5	1	0	2	0	1	9	7
	4	50	17	24	1	3	1	2	4	2	1	13	10
	5	50	33	54	5	2	12	6	1	7	1	29	20
	6	50	31	48	2	2	11	9	4	3	1	30	16
		300	137	225	22	29	38	17	11	12	18	125	78
15-----	1	50	0	0	0	0	0	0	0	0	0	0	0
	2	50	0	0	0	0	0	0	0	0	0	0	0
	3	50	1	1	0	1	0	0	0	0	0	1	0
	4	50	2	2	0	1	0	0	0	0	0	1	1
	5	50	3	3	0	0	0	0	2	0	0	2	1
	6	50	11	12	0	3	1	1	2	1	0	8	4
		300	17	18	0	5	1	1	4	1	0	12	6
16-----	1	50	50	321	8	22	14	13	34	31	32	146	167
	2	50	28	59	3	2	0	3	4	12	3	24	32
	3	50	19	19	0	4	0	0	0	1	1	6	13
	4	50	41	80	6	9	1	16	9	9	7	51	23
	5	50	39	58	0	21	4	7	4	1	1	38	20
	6	50	48	138	0	57	18	18	5	2	1	101	37
		300	225	675	17	115	37	57	56	56	45	366	292
17-----	1	50	40	103	8	5	9	7	8	11	12	52	43
	2	50	40	80	2	22	0	0	4	1	0	27	51
	3	50	34	53	1	5	0	0	0	1	0	6	46
	4	50	30	44	1	15	0	2	1	0	1	19	24
	5	50	34	47	4	17	8	1	1	0	1	28	15
	6	50	41	91	0	13	4	1	6	6	0	30	61
		300	219	418	16	77	21	11	20	19	14	162	240
18-----	1	50	14	18	0	4	1	5	0	0	0	10	8
	2	50	19	23	1	1	1	2	1	0	0	5	17
	3	50	9	10	0	0	1	1	2	4	0	8	2
	4	50	11	11	0	2	2	5	1	1	0	11	0
	5	50	7	8	0	1	2	1	1	2	0	7	1
	6	50	6	7	0	1	0	1	2	1	0	5	2
		300	66	77	1	9	7	15	7	8	0	46	30
19-----	1	50	44	177	18	24	22	12	39	37	21	155	4
	2	50	19	33	5	2	6	1	6	3	3	21	7
	3	50	15	21	4	3	1	3	3	1	2	13	4
	4	50	3	3	1	0	0	1	0	1	0	2	0
	5	50	3	7	5	1	0	3	0	1	0	5	-----
	6	50	8	9	1	3	0	1	3	0	1	8	0
		300	92	250	34	33	29	21	51	43	27	204	15
20-----	1	50	4	4	0	1	1	1	0	1	0	4	0
	2	50	0	0	0	0	0	0	0	0	0	0	0
	3	50	3	3	0	1	1	0	1	0	0	3	0
	4	50	5	5	1	1	0	0	1	0	1	3	1
	5	50	13	17	1	3	3	0	4	0	1	11	5
	6	50	13	15	0	4	5	0	3	1	0	13	2
		300	38	44	2	10	10	1	9	2	2	34	8
21-----	1	50	49	226	8	36	35	18	37	11	1	138	80
	2	50	45	131	0	45	29	6	14	1	0	95	36
	3	50	35	60	0	38	2	1	3	0	0	44	16
	4	50	24	29	0	24	0	0	0	0	0	24	5
	5	50	28	35	0	14	0	0	1	0	0	15	20
	6	50	13	15	0	3	0	0	2	0	0	5	10
		300	194	496	8	160	66	25	57	12	1	321	167

## WAREHOUSE No. 2

The 30,000 sacks of chick-peas stored in this two-story brick warehouse were stacked on both floors, each floor measuring 150 by 50 by 10 feet, with wooden floors, and with large sliding doors on the ground floor opening on the street on either side of the warehouse. Both floors were fumigated as a single unit. The sacks were stacked from 8 to 10 deep. The sacks sampled were on the ground floor and located as indicated in figure 1, *b*, being well toward the rear of the warehouse between a side aisle and a brick wall, the tier A being 15 rows or tiers from the street wall. Sacks 1 to 6 were in tier B; sacks 7 to 10 in tier C; sacks 11 to 18 in tier A. Tiers of sacks on either side of a block A-B-C, and a portion of the sacks of tier A were removed to make possible the taking of samples. While this warehouse was well constructed, considerable leakage of gas took place through the paper pasted over the iron gratings above the doors. It is believed by the writers that sacks 11 to 18 stacked against the rear brick wall were located in as difficult a position as any from the standpoint of gas penetration. The results of the examination of the samples taken in warehouse No. 2 are recorded in Table II.

TABLE II.—*Data on the penetration of hydrocyanic-acid gas into 240-pound sacks of chick-peas infested by Bruchus quadrimaculatus and arranged as shown in figure 1, b. All bruchids reached and killed by the fumigant*

Sack No.	Sample No.	Number of seeds examined	Number of seeds infested	Number of—								Total larvæ, pupæ, and adults	Eggs unaccounted for
				Eggs	Emergence holes	Larvæ				Pupæ	Adults		
						One-eighth	One-fourth	One-half	Full-grown				
1.....	1	50	49	308	38	3	5	14	9	16	17	64	206
	2	50	42	191	3	4	7	5	9	31	28	84	104
	3	50	27	67	5	13	1	5	9	8	2	38	24
	4	50	20	26	0	1	1	1	1	0	0	4	22
	5	50	9	11	0	0	0	0	0	0	0	0	11
	6	50	4	6	0	2	0	0	0	0	0	2	4
		300	151	609	46	23	14	25	28	55	47	192	371
2.....	1	50	48	243	2	22	12	5	5	3	10	57	184
	2	50	46	353	7	2	0	4	17	4	28	55	291
	3	50	48	328	4	31	0	16	13	21	31	112	212
	4	50	49	327	4	7	3	16	7	27	15	75	248
	5	50	49	444	5	173	66	20	25	17	5	306	133
	6	50	23	44	0	5	1	5	9	3	5	28	16
		300	263	1,739	22	240	82	66	76	75	94	633	1,084
3.....	1	50	50	282	16	38	3	15	19	29	2	106	160
	2	50	41	104	3							101	
	3	50	16	28	0	9	3	3	1	14	0	30	
	4	50	5	5	0	2	2	1	0	0	0	5	0
	5	50	2	2	0	0	2	0	0	0	0	2	0
	6	50	10	13	0	4	0	2	1	1	0	8	5
		300	124	434	19	53	10	21	21	44	2	151	266
4.....	1	50	42	124	2	17	3	6	30	10	6	72	50
	2	50	40	104	4	13	11	10	24	2	6	66	34
	3	50	36	76	2							74	
	4	50	39	82	1	19	2	4	1	2	6	34	47
	5	50	30	49	4	16	1	0	2	2	2	23	22
	6	50	25	38	1	10	1	1	0	1	1	14	23
		300	212	473	14	75	18	21	57	17	21	209	250
5.....	1	50	6	5	0	1	0	0	1	1	1	4	1
	2	50	1	3	0	0	0	0	1	1	0	2	1
	3	50	2	3	0	0	0	0	0	0	0	0	3
	4	50	3	3	0	0	0	0	0	1	0	1	2
	5	50	25	49	0	4	2	4	5	2	1	18	31
	6	50	37	89	4	24	6	1	5	11	4	51	34
		300	74	152	4	29	8	5	12	16	6	76	72

TABLE II.—Data on the penetration of hydrocyanic-acid gas into 240-pound sacks of chick-peas infested by *Bruchus quadrimaculatus* and arranged as shown in figure 1, b. All bruchids reached and killed by the fumigant—Continued

Sack No.	Sample No.	Number of seeds examined	Number of seeds infested	Number of—								Total larvæ, pupæ, and adults	Eggs unaccounted for
				Eggs	Emergence holes	Larvæ				Pupæ	Adults		
						One-eighth	One-fourth	One-half	Full-grown				
6.....	1	50	24	30	1	3	1	0	6	6	3	19	10
	2	50	9	9	0	0	0	0	0	0	0	0	9
	3	50	0	0	0	0	0	0	0	0	0	0	0
	4	50	0	0	0	0	0	0	0	0	0	0	0
	5	50	2	2	0	0	0	0	0	0	0	0	2
	6	50	10	11	0	4	1	0	1	0	0	6	5
		300	45	52	1	7	2	0	7	6	3	25	26
7.....	1	50	43	226	6	20	5	19	46	38	24	152	68
	2	50	29	62	4	4	0	8	24	12	3	51	7
	3	50	22	43	0	26	3	0	1	0	0	30	13
	4	50	38	70	2	51	4	5	2	0	0	62	6
	5	50	47	232	1	72	1	0	1	0	1	75	156
	6	50	48	343	5	59	7	34	60	20	13	193	145
		300	227	976	18	232	20	66	134	70	41	563	395
8.....	1	50	49	290	6	17	10	10	9	2	1	49	235
	2	50	30	66	0	13	4	2	4	1	0	24	42
	3	50	19	25	1	3	1	1	1	2	2	10	14
	4	50	23	31	2	9	0	2	2	1	1	15	14
	6												
		200	121	412	9	42	15	15	16	6	4	98	305
9.....	1	50	45	206	3	11	20	19	17	28	16	111	92
	2	50	50	220	8	59	29	13	12	17	9	139	73
	3	50	14	16	3	2	0	1	0	0	2	5	8
	4	50	5	7	0	0	2	0	0	0	1	3	4
	5	50	9	10	0	0	0	0	0	0	0	0	10
	6	50	27	54	0	9	5	0	5	0	0	19	35
		300	150	513	14	81	56	33	34	45	28	277	222
10.....	1	50	48	353	44	30	9	2	8	84	23	156	153
	2	50	32	98	14	13	5	7	5	31	4	65	19
	3	50	11	15	1	5	4	0	1	5	1	16	-----
	4	50	5	5	1	0	0	2	5	5	4	16	-----
	5												
	6	50	37	106	2	19	6	8	5	5	2	45	59
		250	133	577	62	67	24	19	24	130	34	298	231
11.....	1	50	50	257	15	21	6	31	55	30	25	168	74
	2	50	50	187	6	59	17	10	12	3	3	104	77
	3	50	49	129	2	71	5	5	5	1	3	90	37
	4	50	28	41	0	0	0	0	0	0	0	0	41
	5	50	27	46	0	27	0	0	0	0	0	27	19
	6	50	40	71	0	36	3	0	0	0	0	39	32
		300	244	731	23	214	31	46	72	34	31	428	280
12.....	1	50	22	30	0	16	10	1	0	0	0	27	3
	2	50	14	18	0	0	0	0	1	2	0	3	15
	3	50	4	5	0	1	1	1	0	0	0	3	2
	4	50	2	2	0	0	2	0	0	1	0	3	-----
	5	50	2	2	0	-----	-----	-----	-----	-----	-----	-----	2
	6	50	14	15	0	8	0	1	0	0	0	9	6
		300	58	72	0	25	13	3	1	3	0	45	28
13.....	1	50	49	329	18	51	32	18	7	2	4	114	197
	2	50	42	156	5	61	9	3	0	1	1	75	76
	3	50	22	34	1	22	0	0	1	0	1	24	9
	4	50	9	10	0	6	1	0	0	1	1	9	1
	5	50	13	16	0	4	2	0	0	0	0	6	10
	6	50	20	29	0	-----	-----	-----	-----	-----	-----	-----	29
		300	155	574	24	144	44	21	8	4	7	228	322

TABLE II.—Data on the penetration of hydrocyanic-acid gas into 240-pound sacks of chick-peas infested by *Bruchus quadrimaculatus* and arranged as shown in figure 1, b. All bruchids reached and killed by the fumigant—Continued

Sack No.	Sample No.	Number of seeds examined	Number of seeds infested	Number of—								Total larvæ, pupæ, and adults	Eggs unaccounted for
				Eggs	Emergency holes	Larvæ				Pupæ	Adults		
						One-eighth	One-fourth	One-half	Full-grown				
14-----	1	50	50	333	36	18	62	36	34	11	19	180	117
	2	50	47	211	1	80	9	1	1	0	0	91	119
	3	50	24	43	0	10	4	1	0	0	0	15	28
	4	50	21	27	0	8	0	0	0	0	0	8	19
	5	50	5	6	0	0	0	0	0	0	0	0	6
	6	50	3	7	1	0	0	2	0	0	2	4	2
	300	150	627	38	116	75	40	35	11	21	298	291	
15-----	1	50	50	588	2	137	11	13	6	6	9	182	404
	2	50	47	373	2	82	6	7	3	1	12	111	260
	3	50	50	429	2	63	3	4	4	1	5	80	347
	4	50	49	343	3	51	0	0	1	0	3	55	235
	5	50	48	399	1	88	10	5	0	2	5	110	288
	6	50	49	639	-----	12	15	32	29	40	148	276	363
	300	293	2,771	10	433	45	61	43	50	182	814	1,947	
16-----	1	50	49	311	10	36	28	23	10	25	9	131	170
	2	50	40	90	0	2	0	0	2	0	0	4	86
	3	50	10	16	0	0	0	0	0	0	0	0	16
	4	50	9	14	0	3	1	0	0	0	0	4	10
	5	50	14	26	0	1	2	2	1	1	1	8	18
	6	50	46	171	1	21	11	1	7	2	3	45	125
	300	168	628	11	63	42	26	20	28	13	192	425	
17-----	1	50	46	136	21	1	4	22	58	50	32	167	-----
	2	50	20	54	1	-----	-----	-----	-----	-----	-----	-----	54
	3	50	18	24	2	2	0	1	4	4	3	15	7
	4	50	16	25	0	4	3	0	2	2	1	11	14
	5	50	14	16	0	-----	-----	-----	-----	-----	-----	-----	16
	6	50	25	42	3	11	5	0	1	1	0	18	21
	300	139	297	27	18	12	23	65	57	36	211	112	
18-----	1	50	46	156	16	3	3	8	42	33	22	111	29
	2	50	30	55	0	14	4	1	7	5	2	33	22
	3	50	12	16	0	0	0	1	3	0	0	4	12
	4	50	8	10	0	4	0	0	0	0	0	4	6
	5	50	14	18	0	3	0	0	0	0	0	3	15
	6	50	17	25	0	4	0	0	0	0	0	4	21
	300	127	280	16	28	7	10	52	38	24	159	105	

## WAREHOUSE No. 3

The 30,000 sacks of chick peas stored in this warehouse were stacked as indicated in Plate 1, B. The warehouse had a wooden frame covered with sheets of galvanized iron. The cracks of the building were papered over by professional paper hangers with bill posters. This warehouse was extremely loose in construction, with so many openings that the writers would not have believed that it could be made sufficiently tight even with the aid of professional paperers.

Badly infested sacks were found heating on both floors of this warehouse. While the samples recorded were taken from the second floor, infestations at a of Plate 1, B, were found equally well killed by the fumigation. The sacks shown in figure 1, c, represent one carload of chick-peas. Sacks No. 12 to 35 were exposed at the ends and numbered, on a 13-inch side aisle, between stacks of carload lots, leading from the main 8-foot aisle to the rear wall.

The data of Table III record the number of bruchid eggs and emergence holes found in the samples taken from sacks 12 to 35, and thus, by comparison with



the detailed data of Tables I and II, indicate that the 50 seeds of each sample were in some instances heavily infested. Upon examination all stages of the bruchids were found dead in seeds, and no development occurred in seeds held for subsequent observation.

TABLE III.—Data on the penetration of hydrocyanic-acid gas into 240-pound sacks of chick-peas infested by *Bruchus quadrimaculatus* and arranged as shown in figure 1, c. All bruchids reached and killed by the gas

Sack No.	Sample No.	Number of—		Sack No.	Sample No.	Number of—		Sack No.	Sample No.	Number of—	
		Eggs	Emergence holes			Eggs	Emergence holes			Eggs	Emergence holes
12-----	1	98	4	13	1	97	2	14	1	222	10
	2	94	2		2	52	0		2	155	6
	3	67	2		3				3	118	8
	4	29	0		4	44	1		4	93	8
	5	93	3		5	79	3		5	95	11
	6	164	6		6	101	7		6	111	12
15-----	1	260	14	16	1	94	10	17	1	429	17
	2	94	15		2	72	8		2	228	2
	3	90	4		3	97	7		3	213	3
	4	107	5		4	135	15		4	247	8
	5	110	7		5	159	13		5	291	7
	6	152	0		6	291	39		6	383	12
18-----	1	45	0	19	1	358	43	20	1	528	53
	2	41	1		2	114	5		2	196	2
	3	48	4		3	86	1		3	75	2
	4	73	4		4	47	0		4	42	1
	5	69	1		5	45	0		5	32	1
	6	75	3		6	56	0		6	82	5
21-----	1	54	10	22	1	389	33	23	1	108	2
	2	45	7		2	204	3		2	64	1
	3	42	4		3	45	2		3	98	3
	4	32	3		4	40	6		4	199	2
	5				5	56	3		5	331	3
	6	205	50		6	39	6		6	498	19
24-----	1	315	18	25	1	321	7	26	1	291	4
	2	137	15		2	214	16		2	307	10
	3	58	2		3	128	2		3	146	6
	4	55	2		4	33	2		4	89	2
	5	34	4		5	37	0		5	69	1
	6	47	0		6	41	1		6	47	4
27-----	1	115	12	28	1	313	13	29	1	132	8
	2	98	7		2	300	3		2	84	3
	3	83	2		3	60	2		3	69	2
	4	131	2		4	39	1		4	64	1
	5	277	13		5	72	0		5	157	2
	6	195	6		6	73	0		6	334	27
30-----	1	128	3	31	1	126	1	32	1	292	26
	2	60	2		2	52	1		2		
	3	67	1		3	52	0		3		
	4	104	1		4	30	0		4		
	5	165	4		5	41	0		5		
	6	227	6		6	57	3		6		
33-----	1			34	1	83	1	35	1	201	11
	2	81	2		2	53	0		2	120	8
	3	35	0		3	51	0		3	119	0
	4	37	1		4	78	1		4	76	4
	5	31	0		5	178	7		5	73	5
	6	49	0		6	206	2		6	132	9

INSECTS OTHER THAN BRUCHIDS

The data on infestations recorded above deal only with *Bruchus quadrimaculatus*. In Warehouse No. 4, of brick construction and very tight, about 110 by 66 by 13 feet, the sacks were stacked only four and five deep. The seeds in them contained some infestations by bruchids, but were more interesting because of severe infestations by *Rhizopertha dominica* and much slighter infestations by *Lasioderma serricorne*. The *Rhizopertha* infestations had in certain sacks reduced the seeds to a condition rendering them easily crushed between the fingers. The *Lasioderma* infestations were primary but with never more than one or two larvæ or pupæ in a seed. This warehouse was fumigated in the same manner as Warehouses 1 to 3, with the result that examinations of

the seeds made with the same tedious carefulness revealed no living specimens of larvæ, pupæ, or adults.

In all five warehouses fumigated there were found slight infestations of *Sitophilus oryza*, *Plodia interpunctella*, and *Sitotroga cerealella*. The *Plodia* infestations appeared to be easily killed by the fumigations. Large numbers of the *Sitophilus* and *Sitotroga* were killed, but so many were not that the fumigations, from the standpoint of their control, could not be called a success. It should be said, in passing, that *Sitophilus* and *Sitotroga* infestations are not usual in chick-pea stocks, bruchids being the only really serious pest, aside from the infrequent and devastating attacks of the *Rhizopertha*.

### OBSERVATIONS MADE SUBSEQUENT TO FUMIGATION

Many samples of seeds were taken from all warehouses just previous to fumigation (November 29, 1918). These were examined seven months later (June, 1919). All contained many living adult and immature bruchids and their parasites, except a few of the more heavily infested samples which had become too badly damaged to support a bruchid infestation, with the result that the infestations had burned themselves out.

Of the hundreds of fumigated samples held for observation only one developed living bruchid material. This one sample showed a few living specimens on June 23, 1919. Many of the fumigated samples were held for observation in the laboratory but developed no infestations up to 1923, although conditions were favorable to bruchid development, as indicated by the growth of bruchid cultures in other containers and by the ease with which the fumigated seeds became infested in 1923 when adult bruchids were given access to them.

### SUMMARY

It has been generally conceded that commodities such as wheat, corn, rice, animal feeds, flour, etc., stored in bulk in farmers' bins, grain elevators, or stacked closely in sacks in warehouses, are not satisfactorily protected by ordinary fumigation with hydrocyanic-acid gas because this gas lacks sufficient power of penetration to reach and kill insects hidden within the commodity. Since such food supplies are subject to severe insect attack in storage, usually in congested districts where inflammable and explosive gases can not be used without danger, all data bearing upon the penetration of hydrocyanic-acid gas are of value. Because food commodities in storage under private ownership are of great value, subject to removal on short notice according to trade conditions, and therefore not available for prolonged study, opportunities for studies upon the penetration and effectiveness of insecticide gases are rare and, when offered, should be improved. The data recorded on the penetration and effectiveness of hydrocyanic-acid gas during the fumigation of 137,000 sacks of chick-peas (*Cicer arietinum*) each weighing 240 pounds, the lot having a retail value of \$5,000,000, are new and valuable.

No similar large-scale practical work with a warehoused commodity has previously been recorded. It was found that infestations by *Bruchus quadrimaculatus*, *Rhizopertha dominica*, *Lasioderma serricorne*, and *Plodia interpunctella* could be brought under very satisfactory control by fumigation with hydrocyanic-acid gas, as a result of the thorough penetration of the gas throughout the bulk of the 240-pound sacks, no matter whether these were stacked four or five deep, as chick-peas are ordinarily warehoused, or in piles, the tiers of which were often 18 sacks high. Infestations of *Sitophilus oryza* and *Sitotroga cerealella* were not satisfactorily controlled by one fumigation, though many were killed.

Since *Bruchus quadrimaculatus* is the primary pest of chick-peas, fumigation with hydrocyanic-acid gas, when generated by the use of  $2\frac{1}{2}$  pounds of 98 to 99 per cent pure sodium cyanid per thousand cubic feet of space, was shown to be a dependable method of protecting chick-peas in storage. Fumigation with hydrocyanic-acid gas has now become the standard control in commercial establishments handling this food.

## PLATE 1

A.—Interior view of storage room of Warehouse No. 1. Note abnormal piling of sacks, making gas penetration more difficult, and barrel in tub used as generator for gas.

B.—Interior view of ground floor of Warehouse No. 3. Weevil infestations breaking out under such abnormal conditions were thoroughly controlled, even when the infestations were in sacks at *a* on the floor.

C.—Fairly normal method of storing chick-peas. Note carload lot to left, only two of the tiers of sacks showing, and narrow space between carload lots extending from main aisle to the rear. Man with grain car probe about to take samples from sacks No. 12 to 17, which are the same sacks as those shown in text Figure 1, *c*.



# A NEW NEMATODE, CYLINDROPHARYNX ORNATA, FROM THE ZEBRA, WITH KEYS TO RELATED NEMATODE PARASITES OF THE EQUIDAE<sup>1</sup>

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## *Cylindropharynx ornata*, sp. n.

Two males and one female of this species were found in material collected from *Equus grevyi* at Bethesda, Md., in 1907, one male among specimens collected from the feces by Dr. E. C. Schroeder and the other two among specimens collected post mortem by Dr. M. C. Hall. The worms were collected from one or both of two zebras presented by the King of Abyssinia to Ex-President Roosevelt and turned over by him to the Department of Agriculture.

Size: Male 9.9 mm. in length and 0.4 mm. greatest breadth. Female 10.0 mm. in length and 0.5 mm. greatest breadth.

HEAD—MOUTH COLLAR.—Depressed at margin; marked off from rest of the skin by a constriction; it is considerably higher dorsally and ventrally than laterally.

HEAD PAPILLAE.—Submedian papillae very striking in appearance, a distal leaf-shaped portion being separated by a constriction and bent inward from the tall digitiform proximal portion.

Lateral papillae not conspicuous; do not project beyond mouth opening; their extremities are trifurcated, as noted by Boulenger (1)<sup>2</sup> in *C. brevicauda*.

MOUTH CAPSULE.—This shows the great length characteristic of the genus; in the male 250 $\mu$ , in the female 300 $\mu$  long. The diameter of the anterior third of the capsule is markedly greater than that of the posterior two-thirds, giving the appearance of a bulbous swelling. In the male the diameter of the anterior portion was 140 $\mu$ , of the posterior portion 83 $\mu$ ; in the female 166 $\mu$  and 99 $\mu$  respectively. The ratio of greatest width to length of capsule was therefore 1:1.8 in both sexes. The capsule is lined with the usual transparent chitinous layer, which in the anterior bulbous portion projects into the lumen in what appears as a ring-like thickening. The walls of the mouth capsule are thick, their diameter being 11 $\mu$  in the anterior portion and 16 $\mu$  in the posterior portion.

DORSAL OESOPHAGEAL GUTTER.—This does not project into the buccal capsule.

LEAF CROWNS.—The external leaf crown in this species, as in the three previously described, consists of six elements, all clearly projecting beyond the mouth opening. The four occupying the submedian positions are delicate, relatively small, and sharply pointed leaves; the two which are situated laterally are much broader; their tips are bent toward the exterior. The inner surface of these leaves is grooved, giving the appearance of three ridges with two troughs between them; these are divergent toward the base of the leaf. Boulenger (1) noted that the corresponding leaves of *C. brevicauda* were notched in a similar manner and suggests that this may indicate that the leaf resulted from the fusion

<sup>1</sup> Received for publication Mar. 27, 1924.

<sup>2</sup> Reference is made by number (*italic*) to "Literature cited," p. 672.

of at least two elements. In the present species at least three elements would be indicated from the grooves. Situated in the space between the broad leaf and the corresponding lateral papilla are two accessory elements, which have not been described, from the other species of *Cylindropharynx*. These are very transparent and their structure is ascertained only with difficulty; their position and general shape are shown in Figures 1, 2, and 3. From the dorsal and ventral

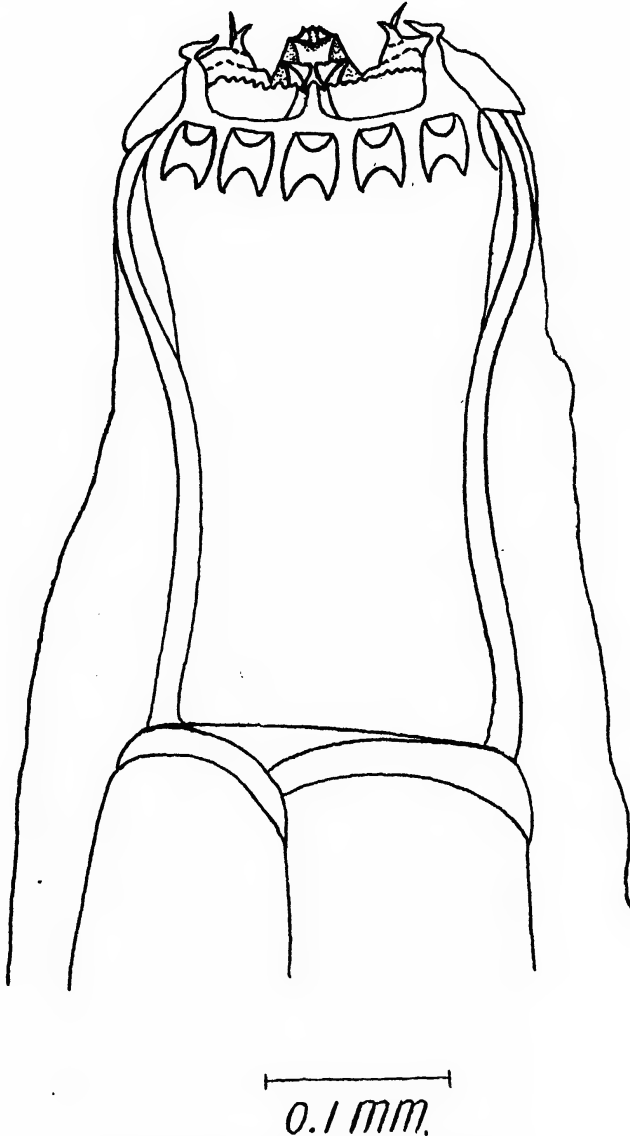


FIG. 1.—Anterior extremity in lateral view

lips of the mouth collar broad plates project into the lumen of the mouth. Their elaborate structure is shown in Figures 2 and 4. Similar plates have been noted by Yorke and Macfie (8) in *C. rhodesiensis* and *C. brevicauda*.

The internal leaf crown consists of 12 strikingly large, broad elements, the distal-free portion of each being bluntly rounded and bent inwards, and the basal attachment to the mouth capsule showing as a prominent crescentic line. A somewhat similar appearance of the attachment of the elements is seen in

*Gyalcephalus capitatus* in what Yorke and Macfie (7) regard as the posterior terminations of the internal leaf crown, but which Ihle (3) considers separate processes of the posterior border of the mouth capsule wall. These internal elements of *Cylindropharynx ornata* originate considerably behind the anterior margin of the mouth capsule, and on reaching that margin are bent inward as described above.

**OEESOPHAGUS.**—Short and broad, in the female having a length of  $747\mu$  and greatest breadth  $216\mu$ ; in the male  $581\mu$  and  $166\mu$  respectively. The nerve ring is situated near the anterior extremity. The excretory pore lies at the union of the posterior and middle thirds of the oesophagus; the cervical papillae are situated a short distance anterior to the pore.

**INTESTINE.**—This shows pseudo-annulations throughout its length, which are especially striking in the anterior part. Leiper (4) indicated this in the figures of *C. brevicauda* and *C. longicauda*.

**POSTERIOR EXTREMITY OF THE MALE.**—The breadth of the worm decreases only very slightly and the dorsal lobe of the bursa is short, so that the posterior end of the worm appears very blunt. The dorsal ray is  $202\mu$  long, tapers to a long, sharp point, and midway from base to tip gives off an external branch, which is bifurcated in its distal half, the inner finger-like process being slightly shorter than the other (fig. 5). There are no fine, hair-like projections on the dorsal rays such as are shown in figures of both *C. rhodesiensis* and *C. brevicauda*. The bursa is finely striated, its margin showing very fine serrations; the lateral lobes do not embrace the genital cone. The genital cone is short ( $100\mu$ ) and stout. The dermal collar is well developed on the ventral but not on the dorsal surface of the cone. The genital appendages are very striking and of very elaborate nature (fig. 6). A central portion, which lies directly under the lip of the genital cone, consists of two globular projections, each bearing a papilla-like point; these two are connected in the middle line by a thumb-like projection. On each side of these central structures there projects a large, irregular-shaped extension, its posterior line wavy, its free end a finger-like process, and on the anterior margin a knob-like projection. These two structures show variation in position, in one male being at right angles to the long axis of the cone (as figured) and in the other the angle being only half that. This variation may indicate that these are clasping organs, therefore motile and capable of changing their relative position. In addition to these complex appendages of the cone, the dermal collar bears an indefinite number of long, delicate, sharply pointed, flexible processes on its lateral and dorsal surfaces. The arrangement of these seems quite irregular. The spicules are long ( $790\mu$ ) and stout, their extremities sharply pointed and strongly barbed (fig. 7).

**POSTERIOR EXTREMITY OF THE FEMALE.**—The vulva is  $1411\mu$  and the anus  $330\mu$  from the tip of the tail. Posterior to the anus there is an abrupt thickening, then the tail tapers to a sharp point. Covering the anus there is a lip-like structure of papilliform appearance (fig. 8).

**DIAGNOSIS.**—This worm is more closely allied to *Cylindropharynx rhodesiensis* than to either of the other two species in the genus, but differs from it in its smaller size, in the much greater width of the mouth capsule in proportion to its length, in the point of origin of the internal leaf crown elements being considerably farther back and in their distal ends being bluntly rounded, in the presence of very elaborate genital appendages, and in the different shape of the spicules. A comparison of the four species of the genus is seen in the key on page 668.

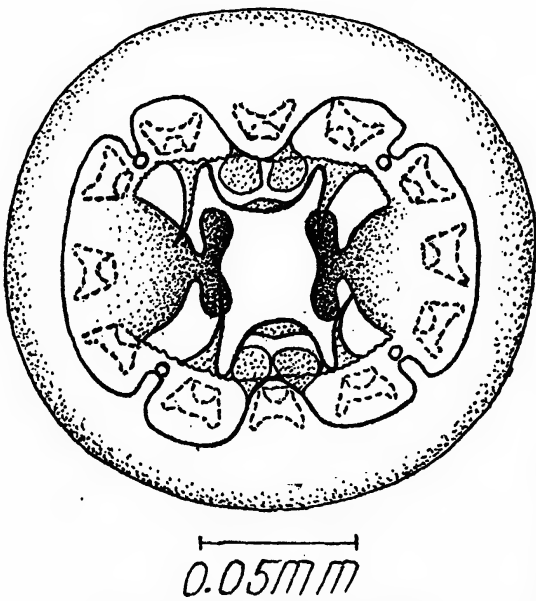


FIG. 2.—Head viewed from front

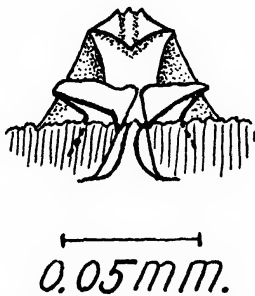


FIG. 3.—Broad external leaf-crown element—  
and secondary structures in front of it

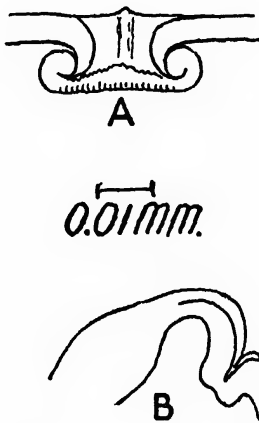


FIG. 4.—Horizontal plate of dorsal and ven-  
tral lips; (A) front view, (B) side view

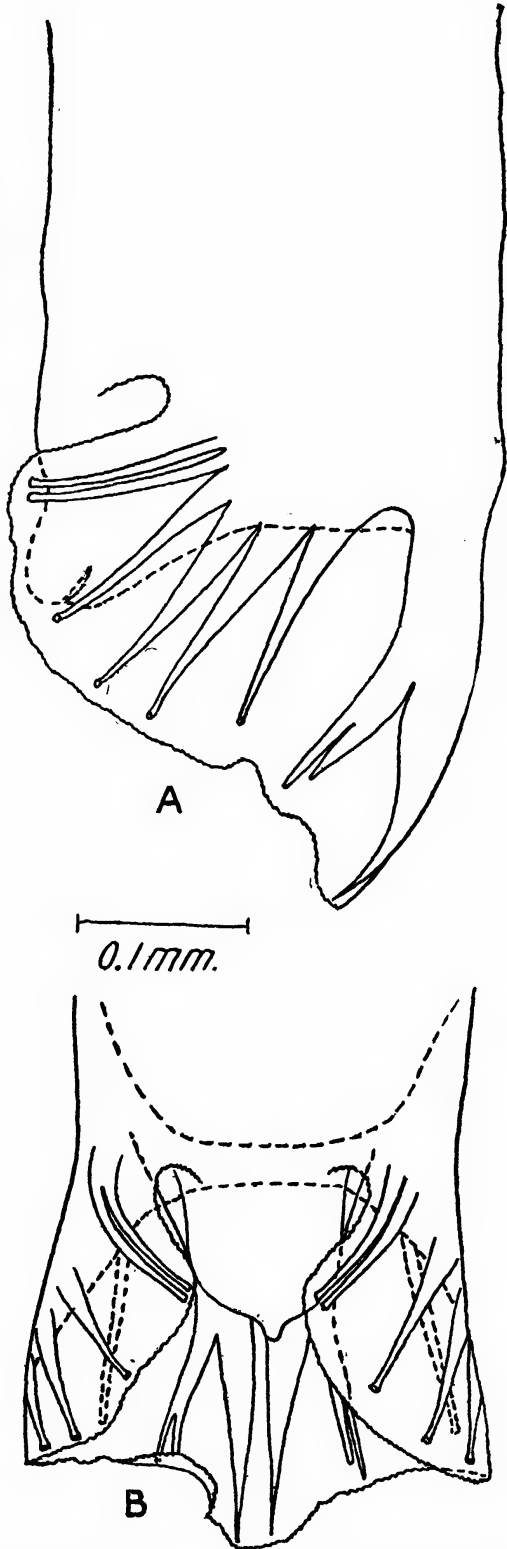


FIG. 5.—Bursa of male; A, lateral view; B, ventral  
view





FIG. 6.—Appendages of genital cone and dermal collar



FIG. 7.—Spicule, lateral view

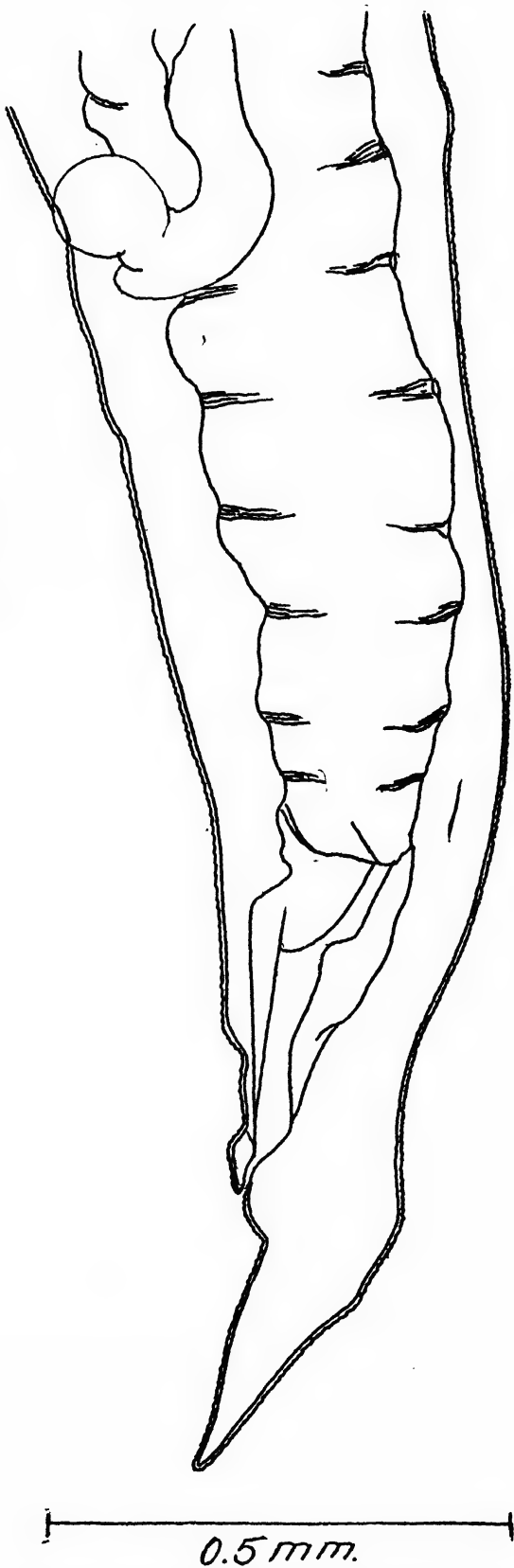


FIG. 8.—Posterior extremity of female, lateral view

## STRONGYLIDAE OF THE HORSE, DONKEY, MULE, AND ZEBRA

## KEY TO THE GENERA.

1. Mouth capsule greatly elongated; external leaf crown contains only six elements and the two occupying the lateral positions are broader than the four occupying the submedian positions... *Cylindropharynx*, p. 668.  
Mouth capsule not greatly elongated; external leaf crown elements usually more numerous than six, and all similar in size and shape.....2.
2. Teeth present in oesophageal funnel.....3.  
No teeth present in oesophageal funnel.....5.
3. Teeth of oesophageal funnel do not project into mouth capsule; bursa bilobed, that is, no median lobe present, and no median dorsal ray; pre-bursal papillae short; spicules terminate without hooks but inclosed in a conspicuous sheath..... *Oesophagodontus*, p. 667.  
Teeth of oesophageal funnel project into mouth capsule; bursa trilobed, that is, a median lobe present and a median dorsal ray; prebursal papillae long; spicules terminate with hooks and without a conspicuous sheath...4.
4. Oesophageal funnel very large and hemispherical, with three pairs of teeth at its base and three large teeth projecting from its walls into the posterior part of the short cylindrical mouth capsule; internal leaf crown elements large and stout; ventral and lateral rays of bursa arise from a common stem..... *Gyalocephalus*, p. 667.  
Oesophageal funnel small and comparatively inconspicuous, possessing three large teeth, each consisting of two plates, which project far into the large, hemispherical mouth capsule; internal leaf crown elements short and relatively inconspicuous; ventral and lateral rays of bursa do not arise from a common stem..... *Triodontophorus*, p. 667.
5. Mouth capsule large, cup or goblet-shaped; dorsal gutter strongly developed, forming a conspicuous thickening along dorsal wall almost or entirely to anterior margin of capsule; teeth may be present as projections of the walls of the capsule in its posterior part.....6.  
Mouth capsule comparatively small, more or less cylindrical; dorsal gutter seldom well developed; walls of mouth capsule never give rise to teeth...7.
6. External leaf crown consists of very numerous slender elements; teeth often, though not always, present in mouth capsule; dorsal and externo-dorsal rays of bursa arise from a common trunk; spicules do not terminate in hooks..... *Strongylus*, p. 667.  
External leaf crown consists of only a few large, broad elements; no teeth in mouth capsule; ventral and lateral rays of bursa arise from a common trunk; spicules undescribed..... *Craterostomum*, p. 668.
7. Internal leaf crown elements large and broad, sometimes not all of equal length; externo-dorsal and dorsal rays arise from a common trunk; this main dorsal trunk split only about half-way to the base, so that the lateral branches arise from the undivided portion; vulva and anus of female comparatively far apart..... *Poteriostomum*, p. 667.  
Internal leaf crown elements vary in size and shape in different species; externo-dorsal and dorsal rays do not arise from a common trunk; main dorsal trunk split more than half way to the base, so that one or both pairs of lateral branches arise from the divided portions; vulva and anus close to each other..... *Cylicostomum sensu lato*, p. 668.

## STRONGYLUS.

1. No teeth in mouth capsule..... *S. edentatus*.  
One or more teeth in mouth capsule.....2.
2. Teeth on both the dorsal and ventral walls of mouth capsule... *S. equinus*.  
Only one bifurcated tooth on dorsal wall of mouth capsule.....3.
3. Male not over 16, female not over 24 mm.; excretory pore at level of nerve ring; the two lobes of the tooth are high and have smooth margins.  
*S. vulgaris*.  
Male 18-32 mm., female 30-43 mm.; excretory pore close behind mouth collar; the two lobes of the tooth low and broad and margins divided into rounded cusps..... *S. asini*.

## TRIODONTOPHORUS.

1. Median lobe of bursa long; teeth of pharynx usually not denticulated.....2.  
Median lobe of bursa short; teeth usually denticulated.....3.
2. Male not over 13, female not over 14 mm. long; mouth collar depressed at margin; vulva 535-700 $\mu$  from posterior end; tail region conical. *T. minor*  
Male 13.5-14, female 16-17 mm. long; mouth collar erect at margin; vulva 270-300 $\mu$  from posterior end; tail region very blunt, obliquely truncated.  
*T. brevicauda*.
3. Tail region of female short; vulva 460-560 $\mu$  from end; spicules 1.25 mm. long; mouth capsule small, length not more than 90 $\mu$ , breadth not more than 140 $\mu$ .....*T. tenuicollis*.  
Tail region of female elongated; vulva 1.4 to 2 mm. or more from end; spicules 3.5-4 mm. long; mouth capsule of moderate size, length 100-130 $\mu$ , breadth 150-180 $\mu$ .....4.
4. Male 14.5-15.5 mm., female 16.5-18.7 mm. long; mouth collar high, circular in profile; vulva 1.4-1.7 mm. from end; spicules have stout barbs. (G. Theiler says this is a synonym of *T. serratus*.)..... *T. intermedius*.  
Male 18 mm., female 25 mm. long; mouth collar low and somewhat depressed at margin; vulva more than 2 mm. from end; spicules do not have barbs.....*T. serratus*.

## GYALOCEPHALUS.

1. Genital cone extends only to free margin of bursa.....*G. capitatus*.  
Genital cone extends beyond margin of bursa; length of cone usually twice as great as preceding species.....*G. equi*.

## OESOPHAGODONTUS.

Males 15-16 mm., females 19-22 mm. long; mouth capsule goblet-shaped; external leaf crown composed of 18 large, projecting elements; internal leaf crown of 36-48 slender elements; dorsal lobe of bursa lacking; postero-lateral ray branched at base; posterior extremity of female straight, gradually tapering from vulva region to tip (a length of 2.3-3 mm.)  
*Oesophagodontus robustus*.

## POTERIOSTOMUM.

1. All the elements of the internal leaf crown of equal size; distance of vulva from anus 925 $\mu$  to 1.5 mm.....*P. ratzii*.  
All the elements of the internal leaf crown not of equal size (2 lateral and 4 submedian elements being longer than the others); distance of vulva from anus 680 to 825 $\mu$ .....2.
2. Seven small leaf elements between each of the elongated ones.  
*P. imparidentatum*.  
Ten small leaf elements between each of the elongated ones.  
*P. pluridentatum*.

## CRATEROSTOMUM.

1. Length 9 mm. or more; internal leaf crown consisting of not more than 16 elements; oesophagus over  $500\mu$  long; vulva 1.27 mm. or more from tip of tail.....*C. acuticaudatum*.  
Length not over 8 mm.; internal leaf crown consisting of 18 or more elements; oesophagus not over  $400\mu$  long; vulva not more than 1.1 mm. from tip of tail.....2.
2. Females 8 mm. long; internal leaf crown consisting of 24-25 elements; anus  $425-535\mu$ ; vulva  $905-1100\mu$  from tip of tail .....*C. mucronatum*.  
Females 4-5.5 mm. long; internal leaf crown consisting of 18 elements; anus  $250-270\mu$ , vulva  $530-570\mu$ , from tip of tail.....*C. tenuicauda*.

## CYLINDROPHARYNX.

1. Worms more than 12 mm. long; length of mouth capsule more than  $3\frac{1}{2}$  times its greatest width; no genital appendages.....*C. rhodesiensis*.  
Worms less than 12 mm. long; length of mouth capsule not greater than  $3\frac{1}{2}$  times the greatest width; genital appendages present.....2.
2. Length of mouth capsule from 3 to  $3\frac{1}{2}$  times its greatest width; genital cone long (up to  $400\mu$ ), extending considerably beyond margin of bursa; vulva of female not more than  $750\mu$ , anus not more than  $200\mu$  from tip of tail.....*C. brevicauda*.  
Length of mouth capsule less than 3 times its greatest breadth; genital cone short, not extending beyond margin of bursa; vulva of female more than  $750\mu$ , anus more than  $200\mu$  from tip of tail.....3.
3. Worms not over 7 mm. long; length of mouth capsule more than twice its greatest width; extremity of lateral branch of dorsal ray undivided or with only a very small projection; appendages a simple pair of stout, finger-shaped processes.....*C. longicauda*.  
Worms over 7 mm. in length; length of mouth capsule less than twice its greatest width; extremity of lateral branch of dorsal ray deeply bifurcated; appendages elaborate structures on genital cone and on dermal collar.....*C. ornata*.

## CYLICOSTOMUM SENSU LATO.

Ihle (2) has divided the large number of species previously placed under *Cylicostomum* Railliet into seven groups, to five of which he gives the rank of subgenera. I prefer to raise these to genera and to give the two remaining groups generic names, believing that this will simplify matters. For the Montgomery group of Ihle I propose the name *Cylicotoichus*, and for his *Brevicapsulatum* group the name *Cylicobrachytus*. The latter group contains only two species; further study may show that they do not belong in the same genus, but until that time confusion will be avoided by placing the group in a position corresponding with that of the other groups which were made by Ihle.

1. Dorsal and ventral walls of mouth capsule much higher (that is, longer in the direction of the long axis of the worm) than lateral walls. *Cylicotoichus*.  
Mouth capsule radially symmetrical (that is, wall of equal height throughout, or without great difference in height). (See *C. brevicapsulatum*).....2.
2. Mouth capsule extremely short (may be as high as  $13-14\mu$  in *C. prionodes*); elements of internal leaf crown either inconspicuous or wanting.  
*Cylicobrachytus*.  
Mouth capsule not extremely short (may be as little as  $17\mu$  high in *C. labratum*).....3.
3. Posterior end of female strongly bent dorsally, with a swelling before the vulva, the tail in lateral view resembling a human foot. *Cylicocercus*.  
Posterior end of female straight or slightly bent dorsally.....4.

4. Internal leaf crown elements extremely large.....*Cylicodontophorus*.  
Internal leaf crown elements small.....5.
5. Internal leaf crown elements originate at some distance behind anterior margin of the mouth capsule.....*Cylicostomum sensu stricto*.  
Internal leaf crown elements originate close to anterior margin.....6.
6. Mouth capsule with hoop-like thickening at posterior margin.. *Trichonema*.  
No hoop-like thickening.....7.
7. Mouth capsule outline mostly rectangular or trapezium-shaped in lateral or dorso-ventral view; posterior end of female mostly straight.  
*Cylicostephanus*.

## CYLICOTOICHUS.

Males 4.3–6 mm., females 4.5–6.5 mm. long; dorsal and ventral walls of mouth capsule  $32\mu$ , lateral walls  $22\mu$  high; external leaf crown consists of 18 unusually long pointed leaves; dorsal lobe of male bursa of medium length; tail of female very short and finely pointed; only one type species.  
*C. montgomeryi*.

## CYLICOBRACHYTUS.

1. Male 11.5, female 13.5 mm. long; external leaf crown consists of 45 slender elements; oesophagus comparatively short (in female  $635\mu$ ).  
*C. brevicapsulatum*.
2. Females only described, 10–11.5 mm. long; external leaf crown consists of 24–26 broad-based elements; oesophagus long ( $800$ – $935\mu$ ); type of genus.  
*C. prionodes*.

## CYLIOCERCUS.

1. Genital cone very long (about  $664\mu$ ); elements of external leaf crown number about 48.....*C. mettami*.  
Genital cone not elongated; elements of external leaf crown number not over 30.....2.
2. Three triangular teeth projecting into mouth capsule from walls of oesophageal funnel.....3.  
No teeth projecting into mouth capsule.....4.
3. Appendages of genital cone absent, but posterior part of collar bears two sharply pointed processes directed toward each other...*C. tridentatum*.  
Appendages of genital cone present as thin, delicate plates, with two slender, finger-like processes.....*C. goldi*.
4. Median lobe of bursa fairly long; elements of the external leaf crown very slightly if at all projecting out of mouth opening.....*C. alveatum*.  
Median lobe of bursa extremely short, almost semicircular; elements of leaf crown clearly projecting out of mouth opening.....5.
5. Walls of mouth capsule (ventral view) have convex outer surface; inner surface undulating to give two notches, the posterior, which is the deeper, being the site of insertion of inner leaf crown; appendages of genital cone consist of slight elevations, with three conical processes, the innermost bearing a delicate finger-like projection.....*C. pateratum*.  
Walls of mouth capsule (ventral view) curve concavely on outer surface, convexly on inner; appendages do not have three conical processes...6.
6. Appendages of genital cone moderately long, outer edges notched, ends rounded; no elongate or digitate processes.....*C. catinatum*.  
Elongate or digitate processes on appendages of genital cone.....7.
7. Each appendage consists of two long, slender, finger-like processes, each with a short projection on its inner surface and with a short projection on each side between the two processes...*C. catinatum* var. *litteraureum*.  
Each appendage consists of a slight elevation, with two simple, unbranched conical processes.....*C. pseudocatinatum*.

## CYLICODONTOPHORUS.

1. Internal leaf-crown possesses dissimilar elements, 12 of the 46 elements being enlarged and projecting farther than those of the external leaf crown.  
*C. ultrajectinum.*
- All elements of internal leaf crown similar-----2.
2. Dorsal gutter projects into mouth capsule, almost to its anterior border; median lobe of bursa elongated, triangular----- *C. bicoronatum.*
- No dorsal gutter; median lobe of bursa of moderate size-----3.
3. Female 14 mm., male 9-10 mm.; outer leaf crown 60, inner 40-46 elements; posterior end of female suddenly falls away behind the anus to form a short tail; genital cone of male small----- *C. ihlei.*
- Female 6-8, male 6-7 mm.; outer leaf crown 40, inner 30-34 elements; posterior end of female tapers very gradually to a point, the axis always remaining the same as that of body; genital cone of male relatively enormous----- *C. euproctus.*

## CYLICOSTOMUM sensu stricto.

1. Dorsal oesophageal gutter does not extend along wall of mouth capsule---2.
- Dorsal oesophageal gutter does extend along wall of mouth capsule-----3.
2. Mouth capsule short (17-18 $\mu$ ), wider than long, walls of moderate thickness; median lobe of bursa short and pointed----- *C. labratum.*
- Mouth capsule fairly deep (33 $\mu$ , Ihle says 37-41 $\mu$ ), longer than wide, walls remarkably thick; median lobe of bursa long and slender, its sides almost parallel----- *C. coronatum.*
3. Walls of mouth capsule converge from before backwards, are irregular of outline, kneed at least twice on both inner and outer surface.  
*C. aegyptiacum.*
- Walls straight; outline not as irregular as above-----4.
4. Females 12-12.5, males 10-11 mm. long; internal leaf crown of 70-80 elements; median lobe of bursa long and slender----- *C. sagittatum.*
- Females not over 11, males not over 9 mm.; internal leaf crown of not more than 50 elements; median lobe of bursa short-----5.
5. Elements of external leaf crown project  $\frac{1}{2}$  their height; dorsal oesophageal gutter extends  $\frac{1}{3}$  depth of capsule; lateral prominences of posterior extremity of female well developed----- *C. ornatum.*
- Elements of external leaf crown only very slightly projecting; dorsal gutter forms short, blunt tubercle on floor of capsule; lateral prominences of posterior extremity of female not well developed-----6.
6. Appendages of male genital cone absent, replaced by two pointed prominences on posterior part of dermal collar; distance from vulva to anus of female 150 $\mu$ ----- *C. labiatum.*
- Dermal collar of male has prominences like those of *C. labiatum*, but the posterior part of genital cone also has finger-like processes on both sides, varying in number and form; distance from vulva to anus of female 95 $\mu$ .  
*C. labiatum* var. *digitatum.*

## TRICHONEMA.

1. Males 16-17 mm., females up to 26 mm.; mouth collar strongly enlarged in lateral sectors, relatively low in dorsal and ventral sectors; excretory pore posterior to origin of intestine by a distance equivalent to half the length of the oesophagus----- *T. auriculatum.*
- Males not over 14 mm.; females not over 17 mm.; mouth collar of approximately the same height in all sectors; excretory pore either at junction or anterior to junction of oesophagus and intestine----- 2.
2. External leaf crown elements short and flat, their free extremities rounded and not extending to the anterior margin of the collar----- *T. radiatum.*

- External leaf crown elements not short and flat, their free extremities pointed and extending to or beyond the anterior margin of the collar. 3.
3. Dorsal gutter present..... 4.  
Dorsal gutter not present..... 6.
4. Internal leaf crown elements (52 in number) quite large and broad; dorsal gutter short, just extending above base of capsule; prebursal papillae very long ( $250\mu$ )..... *T. adersi*.  
Internal leaf crown elements minute plates; dorsal gutter well developed, terminating about midway between anterior and posterior margin of capsule; prebursal papillae short (not more than  $70\mu$ )..... 5.
5. Males 8–10 mm.; females 9–14 mm.; accessory rays of bursa vary.  
*T. nassatum*.  
Males 6.5–7.5, females 8.2–9.7; median dorsal ray of bursa has constant accessory side branch..... *C. nassatum* var. *parvum*.
6. Excretory pore at junction of oesophagus and intestine.... *T. tetracanthum*.  
Excretory pore anterior to junction of oesophagus and intestine..... 7.
7. Males 6, females 7–9 mm.; external leaf crown consists of 20–24 elements; buccal capsule  $27\mu$  deep; distance between anus and vulva  $81\mu$ .  
*T. leptostomum*.  
Males 12.3–13, females 12.3–17 mm.; external leaf crown consists of 30 or more elements; buccal capsule  $38\mu$  or more deep; distance between anus and vulva  $180\mu$  or more..... 8.
8. Males and females 12.3 mm.; external leaf crown elements project only very slightly from mouth opening and their free extremities are drawn in toward each other; median lobe of bursa of medium length (posterior ray  $635\mu$ ) and fairly broad..... *T. triramosum*.  
Males 13, females 17 mm.; external leaf crown elements project considerably out of mouth opening and curve outward; median lobe of bursa long (posterior ray  $847\mu$  or more)..... 9.
9. Posterior ray of median lobe of bursa about  $847\mu$ ..... *T. elongatum*.  
Posterior ray of median lobe of bursa about 1.5 mm.  
*T. elongatum* var. *kotlani*.

## CYLICOSTEPHANUS.

1. External leaf crown consists of not more than 8 elements; dorsal gutter extends along the mouth capsule almost to anterior margin..... 2.  
External leaf crown consists of from 14–35 elements; dorsal gutter projects only slightly or not at all along mouth capsule..... 3.
2. Males 6–6.5, females 8 mm. long; oesophagus shows well-marked, ring-like enlargement at its anterior end; median lobe of bursa narrow and long (dorsal ray about  $525\mu$ ); posterior extremity of female straight.  
*C. calicatum*.  
Males 4–4.6 mm., females 4.1–5.6 mm. long; oesophagus does not show ring-like enlargement at anterior end; median lobe of bursa broad and short (dorsal ray  $140$ – $198\mu$ ); posterior extremity of female usually has S-shaped bend..... *C. minutum*.
3. Mouth capsule very deep (about  $70\mu$ ); external leaf crown consists of about 35 elements; oesophagus very long (about 0.8 mm.), tail of female long (anus 0.3 mm. from tip)..... *C. poculatum*.  
Mouth capsule comparatively shallow ( $17$ – $32\mu$ ); external leaf crown consists of 14–18 elements; oesophagus not elongated (0.2 to 0.4 mm.); tail of female comparatively short (anus 0.05–0.1 mm. from tip)..... 4.
4. Males 4.8–5.5 mm., females 4.7–5.7 mm. long; dorsal lobe of male bursa narrow, tapering and very long; anus of female  $95$ – $124\mu$  from tip of tail..... *C. longibursatum*.  
Males 9–9.5 mm., females 10–10.5 mm. long; dorsal lobe of male bursa short and broad; anus of female  $56\mu$  from tip of tail..... *C. hybridum*.

## ADDENDUM

Since the writing of this paper Railliet (5) has published a description of *Strongylus tetracanthus* Mehlis and has shown that it is identical with *Trichonema arcuata* Cobbold and with *Cylicostomum insigne* Boulenger. The name of the species therefore becomes *Trichonema tetracanthum* (Mehl, 1831) Railliet and Henry, 1919. *Cyathostomum tetracanthum* Looss is a different species and Railliet has named it *Trichonema aegyptiacum*.

Since in raising Ihle's subgenera to genera, *C. insigne* Boulenger was referred to the genus *Cylicocylus*, the latter will fall as a synonym of *Trichonema*. *Cylicostomum*, with the type *C. aegyptiacum*, is therefore available as a generic name for species of the restricted genus *Cylicostomum* as used in this paper, and equivalent to the subgenus *Cylicostomum* as used by Ihle.

Railliet (6) has divided the genus *Strongylus* into four subgenera. One of these, *Decrusia*, contains the strongyle of the elephant. The three subgenera of the horse strongyles, and the distinguishing characteristic of each are as follows: *Strongylus*, two subventral and two dorsal teeth; *Alfortia*, no dorsal teeth; *Delafondia*, two dorsal teeth.

Accordingly the four species are *Strongylus equinus*, *Str. (Alfortia) edentatus*, *Str. (Delafondia) vulgaris*, *Str. (Delafondia) asini*.

Gertrud Theiler in a thesis published in 1923, which has just come to hand, has described a new species of *Cylindropharynx*, *C. intermedia*, from the South African Zebra. It is distinguishable from the other four species as given in the key in this paper in that the specimens of *C. intermedia* are less than 12 mm. long and the length of the mouth capsule is 4 to 5 times as great as its greatest width.

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# CHROMOSOMES IN MAIZE AND MAIZE RELATIVES <sup>1</sup>

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An understanding of the laws of heredity is essential if improvement of plant breeding is to be controlled intelligently. A comprehensive understanding of the mechanism of inheritance demands definite knowledge of the chromosome number, not only of the forms it is desirable to improve but also of the related species. The present paper presents the results of a study of the chromosome number in several genera of the tribe Tripsaceae made in connection with genetic studies of *Zea mays* and its relatives.

Very early in the investigation it was found that *Euchlaena perennis* had 20 pairs of chromosomes instead of 10, the number found in annual teosinte, *E. mexicana*, and in maize. Since perennial teosinte hybridizes with maize and the cross seems likely to be of much genetic interest, it seems desirable to publish this fact together with other cytological data which have come to light.

The larger part of the material used in this study was from plants growing during the summer of 1923 at Lanham, Md., Arlington, Va., and in one of the department greenhouses at Washington, D. C. The material for the study of the chromosomes of two Mexican Tripsacums was collected in Mexico by Messrs. Collins and Kempton the same season.

Two cytological methods of investigation were used in the study of pollen mother-cell development. The iron-acetic-carmin method outlined by Belling (1)<sup>2</sup> was used while growing material was available and it was found that very splendid mitotic figures were quickly stained and ready for study. To verify the results of this quick method, material was also killed in chromo-acetic or Bouin's solutions, embedded in paraffin, sectioned, and stained in Heidenhain's iron-alum haematoxylin. The former method is very satisfactory for studying chromosomes, for they appear more normal than in material prepared by the second method, but the second method has the advantage of leaving permanent slides as records of the conditions found.

## EUCHLAENA

***Euchlaena perennis* Hitch.** Perennial teosinte.

The material for a cytological study of the pollen mother-cell development of this species was taken from plants collected or from plants grown from seed collected in Mexico at the same time by Collins and Kempton (?). These plants were in one of the department greenhouses, Washington, D. C., and at Lanham, Md. The latter had been transplanted from the greenhouse in April. No difference was apparent in materials from plants growing at these two localities.

The haploid chromosome number in *E. perennis* was found to be 20 (Pl. 1, A and B). Since this species is likely to become more important as it becomes better known by corn experimenters, great care was taken in determining the number and size of the chromosomes. The 20 bivalent chromosomes, as represented in Plate 1, C, are distributed in a regular manner during the heterotypic division.

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<sup>1</sup> Received for publication Apr. 9, 1924.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," pp. 680-681.

**E. mexicana** Schrad. Annual teosinte.

Favorable pollen mother-cells of this species show 10 as the haploid chromosome number (Pl. 2, B, E, and K), corresponding to the number determined by Kuwada (14, 15). Plate 2, H, represents a heterotypic anaphase with 11 chromosomes going to each pole. This number was found in a very small per cent of the cells studied. In the few anaphases found that had more than 10 divided chromosomes the extra chromosome was slow in dividing and the halves lagged in their movement to the poles.

It was found in material collected for this study that the pollen mother-cells were very frequently abnormal in appearance; the mother-cell tissue of an anther was in one mass, with the nuclei dotted here and there through it. This mother-cell mass eventually subdivided into pollen grains, dividing first into polynucleated sections and finally into cells with a single nucleus. It was found that these nuclei contained the reduced number of chromosomes, but it was not determined whether such cells developed into normal pollen grains or not. Kuwada (13) described a very similar abnormality in amber rice popcorn. He is inclined to think that this was due to insufficient nutrition. The author will not attempt an explanation until material grown under more favorable climatic conditions has been studied.

**Zea mays** L.

To the present time four varieties of corn have been examined. All show 10 bivalent chromosomes at diakinesis. Plate 2, D, represents a typical heterotypic metaphase in the pollen mother-cell development of Chinese waxy maize, and Plate 2, A, the same phase in Tepic corn, a very large variety introduced from Mexico.

The author's investigations of corn have failed to show any deviation from 10 as the haploid chromosome number. The presence of 20 chromosomes in perennial teosinte and of 10 in annual teosinte and waxy and starchy corns makes it appear probable that the basic chromosome number in *Euchlaena* and *Zea* is 10. This view opposes that suggested by Kuwada (13), that is, that the number 12 is original for all the races of *Zea mays* and that *Zea mays* is tetraploid and derived from an original form which had probably 6 chromosomes in reduced numbers.

**EUCHLAENA × ZEA****Zea mays × E. mexicana** (F<sub>2</sub>).

The material for this study was taken from a normal and a dwarf plant in an F<sub>2</sub> progeny of a cross between *E. mexicana* from Chalco, Mexico, and maize of the type known as dwarf (10). The chromosome number in each plant is 10, as was determined by Kuwada for a *Euchlaena-Zea* hybrid (14, 15). No irregularity in chromosome distribution was observed in the pollen mother-cell development.

**Zea mays × E. perennis**.

A dozen or more of these hybrids had been made in 1922, and a morphological and cytological study was made of the pollen development in F<sub>1</sub> plants of nearly all of these. Table I shows pollen counts of mature pollen from seven representative F<sub>1</sub> hybrids. These counts were made from pollen stained with chloralhydrate-iodin solution (water 50 cc., chloral hydrate 50 gm., iodine 1 gm., and absolute alcohol 50 cc.). The pollen was taken from anthers just before they opened. At this time grains containing protoplasm, and therefore seemingly viable, stained deep blue or red, due to the presence of starch or erythrodextrin,

while all grains without protoplasm, and therefore sterile, remained unstained. The table also summarizes the chromosome condition found in 20 heterotypic prophases of 9 of these hybrid forms.

TABLE I.—Records of pollen and pollen formation studies in *Euchlaena perennis* × *Zea mays*

Plant name	Plant No.	Pollen counts			Heterotypic prophase chromosome condition			
		Fertile		Sterile	Tri-valent	Biva-lent	Uni-valent	Remarks
		Blue	Red					
<i>Z. mays</i> x <i>E. perennis</i> :								
Female parent—								
Oregon Evergreen.....	Th. 102	290		330				
S. P. I. 51291.....	Th. 103	175		356				
Missouri Cob Pipe.....	Th. 79				4	7	6	
Do.....	Th. 79	118		114	4	6	6	
Crinkly.....	Th. 82	69		119	3	7	7	
<i>E. perennis</i> x <i>Z. mays</i> :								
Male parent—								
Brachytic crinkly tassel seed.....	Th. 87	174		121	1	9	9	
Do.....	Th. 87	174		121	1	9	9	
Pod.....	Th. 88				3	7	7	
Do.....	Th. 88				3	7	7	
Do.....	Th. 88				3	7	7	
Do.....	Th. 88				4	6	6	
Do.....	Th. 88				5	5	5	
Do.....	Th. 88				1	9	8	Shows only 29 units.
Crinkly lineate.....	Th. 92	109		209	2	10	4	
Do.....	Th. 92	109		209	2	10	4	
Dwarf.....	Th. 93				2	3	18	Early prophase.
Brachytic dwarf.....	Th. 94				4	5	8	
Brachytic liguleless ramose.....	Th. 95				3	9	3	
Do.....	Th. 95				4	7	4	
Do.....	Th. 95				7	4	1	
Chinese waxy.....	Th. 96	296	13	96		10	10	
Do.....	Th. 96	296	13	96	2	9	6	

A study of the pollen mother-cell development in these hybrids showed that the attempt to combine the 10 chromosomes from the corn parent and 20 chromosomes from the teosinte parent gave varying counts. The author for some time found it difficult to determine the chromosome number. The difficulty was due to an irregularity in chromosome fusion and also to the fact that the chromosome number for *Euchlaena perennis* had not been determined at this time. Finally it was found that there are three classes of chromosomes at diakinesis (Pl. 1, E, F, and G), trivalents, bivalents, and univalents. It required practice and good material to segregate the chromosomes according to the above classes, but counts at diakinesis and the heterotypic metaphases gave uniformly 30 chromosome units.

Table I shows the condition found in 20 nuclei selected from different hybrids. The figures are taken from drawings made of characteristic and clear heterotypic phases. A minute study of trivalent chromosomes at this stage shows that many have a large part corresponding to a bivalent chromosome and a small, loosely attached portion. In a few cases the trivalent chromosomes seem to be composed of three loosely and temporarily attached chromosomes. The author considers that these loosely attached chromosomes are univalents which for a short period unite with bivalents or with each other to give trivalent or perhaps bivalent chromosomes.

It is 10 univalent chromosomes that seem to cause the irregularities observed in the fusing of the chromosomes, and in their distribution during the reduction divisions. The heterotypic (Pl. 1, H and I) and to a less degree the homotypic (Pl. 1, J) divisions are very characteristic. The bivalent chromosomes split in a normal manner and move toward the poles. This division seems also to include all or nearly all of the trivalent chromosomes, one part of each going toward one pole and two parts toward the other, although the double portion appears to separate at about the time the chromosomes move from the plate; consequently all move as single units to the poles. The univalent chromosomes are found scattered a bit apart from the nuclear plate while the bivalent and trivalent chromosomes are dividing. Following the separation of the bivalents and trivalents the univalents collect at the nuclear plate (Pl. 1, G). By the time the earlier-divided chromosomes arrive at the poles the univalents at the plate move, some toward the one, some toward the other pole (Pl. 1, H), without undergoing division. These undivided univalent chromosomes are frequently too late in their movement toward the poles to be included in the forming daughter nuclei and are left in the cytoplasm to degenerate (Pl. 1, I). A study of the homotypic divisions shows that occasionally chromosomes lag on the spindle, and some of them fail to be included in the daughter nuclei (Pl. 1, K). The outcome of such a meiosis is the very unequal distribution of the 30 chromosome units, seen at the heterotypic prophase, into the four resulting pollen grains.

This type of meiosis resembles that described by Rosenberg (19), Kihara (12), and other authors for hybrids resulting from crosses between species differing in chromosome number.

That the 10 univalent chromosomes, which lag on the spindle of the heterotypic and homotypic anaphases, came from the corn parent is suggested by the pollen study of one of these bigeneric hybrids. The study was made after it had been observed that the hybrids between starchy and waxy strains of maize, as well as hybrids between *Euchlaena mexicana* and waxy maize, produced pollen grains filled with starch and erythrodestrin in a 1:1 ratio. (See Table II.) The pollen of *E. perennis* × Chinese waxy maize showed only 3 or 4 per cent of the grains filled with erythrodestrin. If the 10 corn chromosomes had gone to make up the bivalent chromosome, approximately a 1:1 ratio of pollen grains filled with starch and erythrodestrin would have been expected. But if the 10 corn chromosomes are the univalents that lag on the spindle of the heterotypic and homotypic divisions and some of them fail to be included in the daughter nuclei, the result would be that a small per cent of the pollen would carry chromosomes having the erythrodestrin factor introduced by the corn parent.

TABLE II.—Pollen counts of forms segregating for the starchy and erythrodestrin characters in pollen formation

Plant	Fertile		Sterile	Per cent of red in fertile	Per cent sterile
	Blue	Red	Clear		
Starchy × waxy maize.....	2, 069	2, 046	A few.	49. 7	-----
Waxy maize × <i>E. mexicana</i> .....	421	392	164	48. 2	16. 8
Waxy maize × <i>E. perennis</i> .....	296	13	96	4. 2	23. 7
Starchy coix × waxy coix.....	658	664	472	50. 2	26. 3
S. P. I. 48866.....	277	290	No count.	51. 1	-----
Starchy coix × waxy coix.....	127	402	No count.	76. 0	-----
S. P. I. 48867.....	521	1, 310	2, 582	71. 5	58. 5

**Coix lachryma jobi L.**

Table II also shows interesting pollen counts in two coix plants the pollen of which was segregating for the waxy-starchy character. The most significant thing noticed in these counts was the condition in S. P. I. No. 48867 where the number of grains showing erythrodestrin was double that showing starch. The high per cent of sterility allows for a 1:1 segregation of waxy and starchy grains, but the death rate in starchy grains must have been much higher than in the waxy. The presence of such a large per cent of erythrodestrin grains suggested the possibility that the segregation had been other than a 1:1 ratio as might occur if there were two factors controlling the expression of the character.

The chromosome number was determined for S. P. I. No. 48867, the coix plant showing the unexpected high per cent of grains staining red. The haploid number is clearly 10 (Pl. 2, C, F, G, I, and L). This number had previously been determined for coix by Kuwada (14, 15).

Plate 2, J, was prepared to show the similarity between the 10 chromosomes of the two maize varieties studied and coix. This comparison of the chromosomes of maize and coix is introduced to show that the variation in the size of the individual chromosomes of coix are as great as those in maize. Kuwada (13) considers that a possible origin of the two large chromosomes in all 10-chromosomed corns is through a fusion of two chromosomes derived from a 12-chromosomed ancestor. The writer is of the opinion that 10 is the basic chromosome number for the Tripsaceae, and this view is supported by the similarity between the 10 chromosomes of corn and the 10 chromosomes of coix.

**Tripsacum.**

Tripsacum is less closely related to either *Zea* or *Euchlaena* than these two genera are to each other. Attempts to cross *Zea* and *Tripsacum* (8) have failed thus far. This investigation has revealed a very different chromosome complex in *Tripsacum* from that found in corn or in teosinte.

The haploid chromosome number for all *Tripsacums* examined appears to be 35. Plate 3, C and D, are drawings of a nucleus from *T. dactyloides*. The chromosomes were separated in about two equal portions by the microtome knife, the two sections showing 35 bivalent chromosomes. It is assumed that the fragment at the right in Plate 3, C, was cut from one of the chromosomes of Plate 3, D. Plate 3, E and F, represent the equal distribution of the chromosomes in the pollen mother-cell divisions. Such divisions are characteristic for all *T. dactyloides* material studied.

Plate 3, A and B, represents the condition in two phases of the heterotypic division of pollen mother-cells from *T. laxum* growing at the department greenhouse, Washington, D. C. These plants were grown from seed collected in Salvador. There are 36 chromosomes in the diakinesis pictured, but two of these have the appearance of univalents. The heterotypic anaphase pictured shows the irregular division of the chromosomes; 30 bivalent chromosomes have divided and are at the poles, while 10 univalent chromosomes are at the nuclear plate region in the process of division. The division of lagging univalent chromosomes at this time makes it necessary to study the early reduction phases in order to determine the chromosome number for forms characterized by such a division.

Other drawings (Pl. 3, H and I) show irregularities in the pollen formation of two Mexican *Tripsacums*. The cytological material for a study of these two forms was collected in Mexico by Messrs. Collins and Kempton in the fall of 1923. These figures demonstrate that the irregularities in chromosome distribution during meiosis are not due to greenhouse conditions.

Chromosome counts from pollen mother-cells of all species of *Tripsacum* studied are difficult and extremely so in all except *T. dactyloides*, where the reduction phases are more regular. The author attributes this difficulty in determining the chromosome number either to a lack of fusion of some chromosomes at diakinesis, giving nuclei that have more than 35 chromosomes, or to the combining of more than two chromosomes forming large chromosomes, giving nuclei with less than 35 chromosomes. The chromosome number, however, is too large to allow exact chromosome counts and classifications. Nevertheless a detailed study of pollen mother-cells of all the *Tripsacum* material available in the season of 1923 indicates that the haploid chromosome number is about 35.

## DISCUSSION

The haploid chromosome number for representative species of the outstanding genera of the tribes of *Tripsaceae* (15) and *Andropogoneae* (3, 14, 15) have, with few exceptions, been found to be 10 and multiples of 10.

Kuwada (13) has used the chromosome number found in a few *Zea mays* strains as a basis for his view that *Zea* is a tetraploid species derived from ancestral types having 6 as the basic chromosome number. The author has made a preliminary investigation of the chromosome number in maize and maize relatives. He concludes, from the general occurrence of 10 and multiples of 10 chromosomes in the four genera of the *Tripsaceae* and from the presence of 20 haploid chromosomes in a recently discovered species, *Euchlaena perennis*, that 10 is the basic chromosome number for *Zea mays* and its relatives.

The outstanding species of this study has been *Euchlaena perennis*. The presence of 20 as the reduced chromosome number seems to attract attention toward the supposed close relation of the *Euchlaenas* and *Zea*. A chromosome number for perennial teosinte above that of the annual teosinte and maize makes the apparent relation among the three species difficult to explain. Morphological characters indicate that the annual teosinte and maize are of more recent origin than *E. perennis* and that *Tripsacum* is the least specialized member of this group of grasses. Chromosome numbers of typical representatives of these three genera indicate that the more primitive and less specialized members have more chromosomes than the more recent and highly specialized species.

The recent cytological studies in *Rosa* (3, 18, 22), *Rubus* (16), *Oenothera* (6), *Datura* (4), *Triticum* (21), *Avina* (11), *Hyacinthus* (17), and other polymorphic genera seem in a general manner to indicate that the more primitive and more stable species have a smaller chromosome number than more recent or hybrid forms. Such a conception must be reversed in order to derive *Zea mays* and *Euchlaena mexicana* from *E. perennis* or an *E. perennis*-like form having more than 10 haploid chromosomes, and to connect all these forms with the more primitive *Tripsacums*.

A critical investigation of the above-mentioned cytological studies shows very few hybrids that have a chromosome number differing from the sum of the haploid chromosomes of its parents, and only one case, a haploid *Datura* (4), in which there was a chromosome number smaller than that of its parent.

Rosenberg (20) utilized the occurrence of irregularities in chromosome distribution at meiosis to explain the increase in chromosome number of recently originated *Crepis* forms. This explanation generally applied to other genera should allow for an equal chance that hybrids will be found with a lower as frequently as with a higher chromosome number.

F<sub>1</sub> hybrids between maize and perennial teosinte have 30 chromosomes and at meiosis show a chromosome behavior such as has been generally described for

hybrids resulting from crosses between plants having different chromosome numbers.

The presence of trivalent chromosomes at diakinesis of the pollen mother-cell recalls the studies of Belling (2), in which he reports that, in polyploid forms, the chromosomes combine not only to form bivalent, but also trivalent, tetravalent, and even pentavalent groups. The random distribution of the undivided univalent chromosomes at the heterotypic anaphase of the pollen mother-cell development in this corn-teosinte hybrid is similar to that described by Sax (21) for the  $F_1$  hybrids of *Triticum monococcum*  $\times$  *T. turgidum*.

The possible identification of the univalent chromosomes present at meiosis in *E. perennis*  $\times$  *Zea mays* hybrids is suggested by a study of the pollen where the corn involved was of the Chinese waxy type. The pollen in this cross had only 4 per cent of the grains showing erythrodextrin. There is also a very small percentage of meioses in which there are no extruded chromosomes. This may indicate that the extruded chromosomes are contributed to the hybrid by the waxy corn parent. This is contradictory to the chromosome behavior in the pollen mother-cells of a *Nicotiana* hybrid described by Goodspeed (9), where the bivalents were probably made up by a union of chromosomes from the two parents and the univalents were the leftovers from the parent with the larger chromosome number. The division of the univalent chromosome at the heterotypic division in this *Nicotiana* hybrid is unlike that found in teosinte-corn hybrids and similar to that described by Sax (21) for hybrids between the emmer and the vulgaris groups of *Triticum*.

The study of the *Tripsacums* has shown that in this genus the haploid chromosome number is approximately 35. The reduction divisions of all Mexican forms studied resemble minutely that described by Bremer (5) for several varieties of *Saccharum officinarum*. In the *Tripsacums*, as in *S. officinarum*, chromosomes failed to fuse at diakinesis and the resulting univalents lag on the spindle. The division of lagging univalents occurs in the heterotypic division and this associated with a varying number of univalents in different pollen mother-cells makes exact chromosome counts very difficult.

## SUMMARY

The chromosomes of *Euchlaena perennis* pair promptly at diakinesis of the pollen mother-cell, giving 20 bivalents.

The chromosome number of *Euchlaena mexicana* at diakinesis of normal-appearing pollen mother-cells is 10 bivalents. Occasionally 11 or 12 chromosomes have been found in reduction phases that appeared abnormal. The mother-cell tissue in anthers of this species is frequently in a multinucleated mass and very abnormal in appearance.

The chromosomes of *Zea mays*, strains Tepic and Chinese waxy, pair promptly at diakinesis of the pollen mother-cell, giving 10 bivalents.

The chromosomes of *Zea mays*  $\times$  *Euchlaena mexicana* pair promptly at diakinesis of the pollen mother cell, giving 10 bivalents.

The chromosomes of *Zea mays*  $\times$  *Euchlaena perennis* combine at diakinesis of the pollen mother-cell into trivalent or bivalent chromosomes or remain uncombined as univalents. The number of chromosome elements is 30, the sum of the haploid number of the two parents. The distribution of the 30 chromosome elements at meiosis is very irregular.

The chromosomes of *Coix lachryma jobi* pair promptly at diakinesis of the pollen mother-cell, giving 10 bivalents.

The chromosomes of *Tripsacum dactyloides* pair promptly at diakinesis of the pollen mother-cell, giving approximately 35 bivalents.

In *Tripsacum laxum*, *T. pilosum* and *T. lanceolatum* the presence of both univalent and bivalent chromosomes at diakinesis makes the assigning of a definite haploid chromosome number difficult. This number, nevertheless, is approximately 35.

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## PLATE 1 <sup>a</sup>

A.—*Euchlaena perennis* Hitch. Heterotypic prophase, 19 bivalent and 2 single chromosomes.

B.—*Euchlaena perennis* Hitch. Heterotypic metaphase.

C.—*Euchlaena perennis* Hitch. Heterotypic anaphase.

D.—*Euchlaena perennis* Hitch. Early homotypic metaphase.

E.—*Euchlaena perennis* × *Zea mays*. Th. 9522. Heterotypic prophase, 7 trivalent, 4 bivalent, and 1 univalent chromosome.

F.—*Euchlaena perennis* × *Zea mays*. Th. 9522. Heterotypic metaphase, 3 trivalent, 8 bivalent, and 5 univalent chromosomes.

G.—*Zea mays* × *Euchlaena perennis*. Th. 10322. Heterotypic metaphase, 2 trivalent, 11 bivalent, and 2 univalent chromosomes.

H.—*Euchlaena perennis* × *Zea mays*. Th. 9322. Heterotypic anaphase; a typical irregular chromosome distribution.

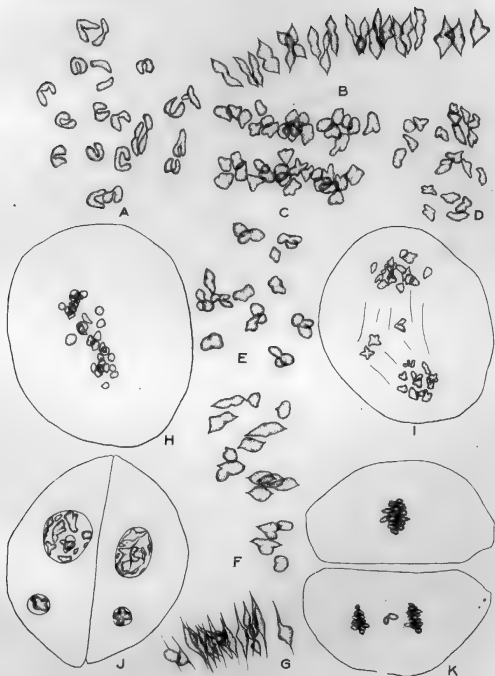
I.—*Euchlaena perennis* × *Zea mays* (pod). Th. 9022. Late heterotypic anaphase.

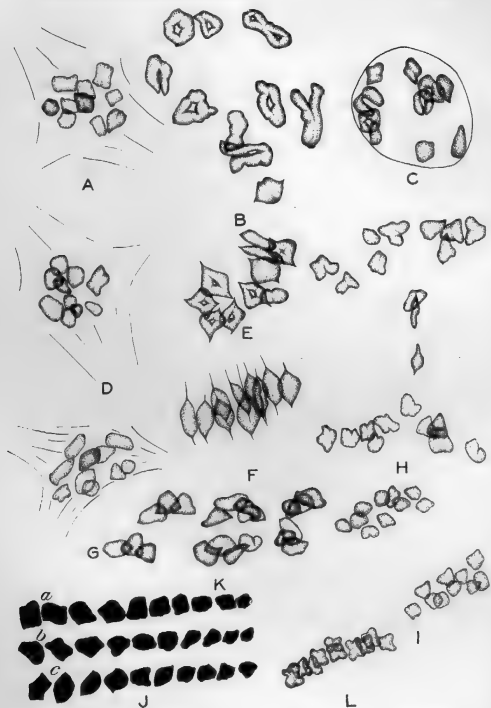
J.—*Zea mays* × *Euchlaena perennis*. Th. 10322. Interkinesis showing two major nuclei and two minor nuclei.

K.—*Zea mays* × *Euchlaena perennis*. Th. 10222. Homotypic divisions.

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\* The plates reproduce camera-lucida drawings of different phases in the reduction divisions of pollen mother-cells of several representative Tripsaceae. A 1.7 mm. oil immersion objective, a No. 12 compensating ocular, and cells stained with iron-acetic-carmin were used in preparing these drawings, except as follows: Heidenhain's iron-alum haematoxylin was used for staining Pl. 1, G to K; Pl. 2, A, D, G; Pl. 3, C and D, E to I. An ocular No. 18 was used for Pl. 2, A, D, G, and for Pl. 3, A. So far as possible, the drawings give details in size and shape of the individual chromosomes, thus allowing a comparison of chromosomes in any particular species or between closely related species. (Reduced one-third in reproduction.)





## PLATE 2

- A.—*Zea mays* L. Tepic. Late heterotypic prophase.  
B.—*Euchlaena mexicana* Schrad. Heterotypic prophase.  
C.—*Coix lachryma jobi* L. S. P. I. No. 48867. Heterotypic prophase.  
D.—*Zea mays* L. Chinese waxy. Late heterotypic prophase.  
E.—*Euchlaena mexicana* Schrad. Heterotypic metaphase.  
F.—*Coix lachryma jobi* L. S. P. I. No. 48862. Heterotypic metaphase.  
G.—*Coix lachryma jobi* L. S. P. I. No. 48867. Late heterotypic prophase.  
H.—*Euchlaena mexicana* Schrad. Heterotypic anaphase.  
I.—*Coix lachryma jobi* L. S. P. I. No. 48867. Heterotypic anaphase with an extra chromosome lagging in its division.  
J.—a. The 10 bivalent chromosomes from Pl. 2, A, arranged according to size.  
b. The 10 bivalent chromosomes from Pl. 2, D, arranged according to size.  
c. The 10 bivalent chromosomes from Pl. 2, G, arranged according to size.  
K.—*Euchlaena mexicana* Schrad. Early heterotypic anaphase.  
L.—*Coix lachryma jobi* L. S. P. I. No. 48862. Homotypic metaphase.

### PLATE 3

A.—*Tripsacum laxum* Nash. Heterotypic prophase, showing 36 chromosomes, 2 of which are probably univalent.

B.—*Tripsacum laxum* Nash. Heterotypic anaphase, having the division of both bivalent and the smaller lagging probably univalent chromosomes.

C and D.—*Tripsacum dactyloides* L. Heterotypic prophase showing 35 bivalent chromosomes.

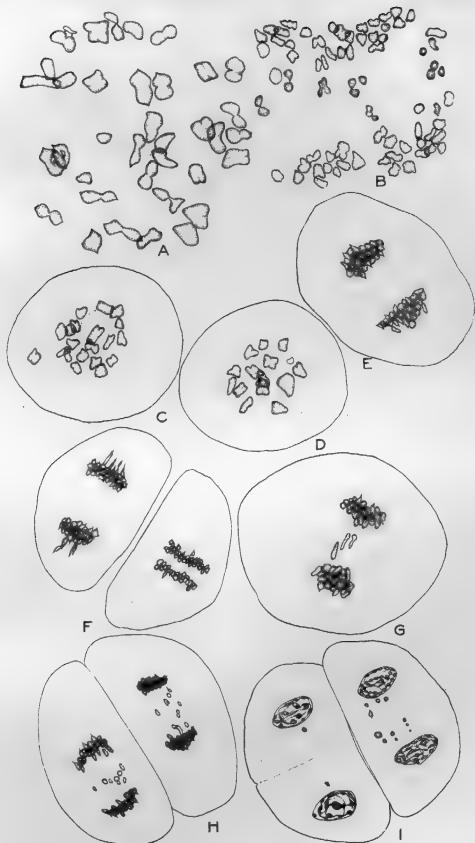
E.—*Tripsacum dactyloides* L. Heterotypic anaphase, showing regular distribution of the chromosomes.

F.—*Tripsacum dactyloides* L. Homotypic anaphase.

G.—*Tripsacum pilosum* Scribn. & Merr. Heterotypic anaphase, showing 3 lagging chromosomes.

H.—*Tripsacum lanceolatum* Rupr. Homotypic anaphase, showing many chromosomes lagging on the spindle.

I.—*Tripsacum lanceolatum* Rupr. Homotypic telaphase, showing extruded chromatin material in the cytoplasm.



# A FUSARIUM BULB ROT OF ONION AND THE RELATION OF ENVIRONMENT TO ITS DEVELOPMENT<sup>1</sup>

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## INTRODUCTION

The *Fusarium* bulb rot of onion was first brought to the attention of the senior writer in 1918 by its occurrence in the large onion-set-growing region in the vicinity of Chicago. It was then one of the three most serious diseases of the onion-set crop, and since that time its importance has increased rather than diminished. A similar disease had been reported from Ohio in 1910 by Selby (8, p. 416)<sup>3</sup>, from Connecticut in 1915 by Clinton (1), and from Japan in 1914 by Hanzawa (4). Since 1918 the reports of *Fusarium* rot of onion in the field as well as in storage and transit have become more numerous and widespread, while the toll of losses due to this disease is rapidly increasing (2, 3, 6).

Laboratory investigations of the disease were begun by the writers in 1921 with material collected in Illinois. Shortly after that time it was learned that Dr. G. K. K. Link was initiating studies upon the disease as it occurred in various other onion-growing regions. Since he was in the better position to assemble and study strains of *Fusarium* on onion from widely different sources, the writers have not attempted to determine whether or not one or more species of *Fusarium* may cause the bulb rot. On the contrary, they have confined themselves to the study of a single strain of *Fusarium* which appears to be the primary cause of the bulb rot in the onion-set sections of Illinois. It is not maintained however that this is the sole cause of the disease in this or other sections, for further survey and study may show other strains or species to be pathogenic on onion bulbs.

This strain belongs to the section *Elegans* and resembles *Fusarium hyperoxysporum* Woll. in some respects and in others it is similar to *F. redolens* and *F. oxysporum*. The description of *Fusarium cepae* by Hanzawa, though not as complete as might be desired, coincides very closely with the organism under discussion, and the symptoms of disease are identical. Therefore the present strain is referred to this binomial and a fuller description of its characters is given.

## DESCRIPTION OF THE DISEASE

Under midwestern conditions this disease appears usually some time after the 1st of July. A progressive yellowing and dying back from the tips of the leaves is the first sign. (See Pl. 1.) The rapidity of this development will vary, sometimes the aerial part dying completely within one or two weeks and in other cases the decay extending over a much longer period. Appearance of newly affected plants may continue until harvest. When the early signs of the disease appear above ground, decay has already started at the stem plate.

<sup>1</sup> Received for publication Feb. 20, 1924.

<sup>2</sup> The writers are indebted to the department of plant pathology, University of Wisconsin, for laboratory and greenhouse facilities placed at their disposal during the course of this investigation.

<sup>3</sup> Reference is made by number (italic) to "Literature cited," pp. 693-694.



The roots commonly turn pink and gradually decay until eventually the entire root system may disappear. A semiwatery decay affecting all the tissues of the succulent scales starts from the base upward. In early infections this decay may continue so as to almost completely destroy the bulb by harvest. In other cases incipient infections at harvest continue to advance during storage and transit, finally leaving dry, shrivelled mummies. In the case of red onions it is common for the anthocyan pigment in the outer epidermis to turn green in color for a considerable distance in advance of the actual decay, indicating a change in reaction of the cell sap. *Fusarium* infection of the bulb is often associated with insect wounds, and since this organism is primarily a wound parasite the coincidence of severe epiphytotics of the disease and maggot injury is common.

## THE CAUSAL ORGANISM

### SOURCE OF PURE CULTURE

The culture of *Fusarium* used in the following experiments was isolated from typically diseased specimens of White Portugal onion sets collected in storage at Morton Grove, Ill. Spore dilution plates were first made, and from a subsequent transfer a pedigreed strain from a single spore was secured. This strain was used for all of the experimental studies.

### MORPHOLOGICAL AND CULTURAL CHARACTERS OF THE ORGANISM<sup>4</sup>

The morphological and cultural characters of the organism as determined from cultures on various media are as follows:

*Fusarium cepae* (Hanzawa) *emend.* Microconidia usually not numerous. Macroconidia gradually attenuate toward the apex, distinctly pedicellate and uniformly curved throughout; typically 3-septate, 35 by 4 (21 to 47 by 3 to 5) microns; from small to medium sporodochia (up to 1.5 mm. in diameter), often converging into pionnotes; chlamydospores intercalary and terminal and commonly present in conidia; aerial mycelium typically scant; when present, short, white to gray; color of conidia from light brown<sup>5</sup> when grown in the dark, to ochraceous salmon or light ochraceous buff when grown in diffuse light; no sclerotia have been observed on any medium; color of substratum quite variable, on slightly acidified hard potato agar plates in diffuse light, from dark vinaceous purple to vinaceous purple; another series on same medium under same conditions color from chocolate to Hessian brown.

Measurements of spores on the various media are as follows:

On hard lima bean agar culture, 9 days old, conidia from pionnotes: Mostly 3-septate, range 21 to 44×3 to 5 $\mu$ , average 35.6×4 $\mu$ .

On hard lima bean agar, culture 20 days old, conidia from pionnotes: Mostly 3-septate, range 30 to 47×4 to 4.6 $\mu$ , average 40×4.3 $\mu$ .

On potato stem plug, culture 21 days old, conidia from small sporodochium: Mostly 3-septate, range 26 to 42×3 to 5 $\mu$ , average 34×3.9 $\mu$ .

On raspberry cane plug, culture 24 days old, conidia from small sporodochium: Mostly 3-septate, range 30 to 38×3 to 5 $\mu$ , average 34.5×4 $\mu$ .

On oat agar, culture 10 days old, conidia from large sporodochium: 75 per cent, 3-septate, range 33 to 44×3 to 4.5 $\mu$ , average 35.7×3.7 $\mu$ ; 24 per cent, 4-septate, range 35 to 45×3.2 to 4.8 $\mu$ , average 39.2×3.6 $\mu$ .

On hard oat agar, culture 250 days old, chlamydospores (in conidia): Abundant, 0-septate, range 7.6 to 10.5×5 to 8.8 $\mu$ , average, 8.2×7.9 $\mu$ .

<sup>4</sup> The writers are indebted to Miss Helen Johann for many helpful suggestions in connection with these studies.

<sup>5</sup> Designations of color are made according to Ridgway (7).

On hard oat agar, culture 130 days old, chlamydospores (in conidia): 0-septate, range 6 to  $10.5 \times 5.5$  to  $8.9\mu$ , average  $8.3 \times 8\mu$ .

On onion agar, culture 275 days old, chlamydospores (in conidia): 0-septate, range 6.7 to  $9.6 \times 6$  to  $9.2\mu$ , average  $7.9 \times 8.4\mu$ .

On malt agar, culture 80 days old, chlamydospores, terminal and intercalary, very variable in size and shape from spherical to ovoid, mostly 1-celled, occasionally 2-celled; 1-celled range, 5.7 to  $9 \times 5.7$  to  $9\mu$ , average  $7.5 \times 7.5\mu$ ; 2-celled, range, 7.5 to  $10 \times 12$  to  $13.5\mu$ , average  $8.0 \times 12.5\mu$ .

After comparison with the descriptions by Wollenweber (10) and Sherbakoff (9) it is evident that this form belongs to the section *Elegans* of the genus *Fusarium* and within that group lies very close to *Fusarium redolens* Woll., *F. hyperoxysporum* Woll. (*F. lutulatum* Sherb.) and *F. oxysporum* Schlecht. It differs from the first of these chiefly in the type of spore curvature. Its macroconidia are uniformly curved throughout (fig. 1), while a distinguishing character of *F. redolens*

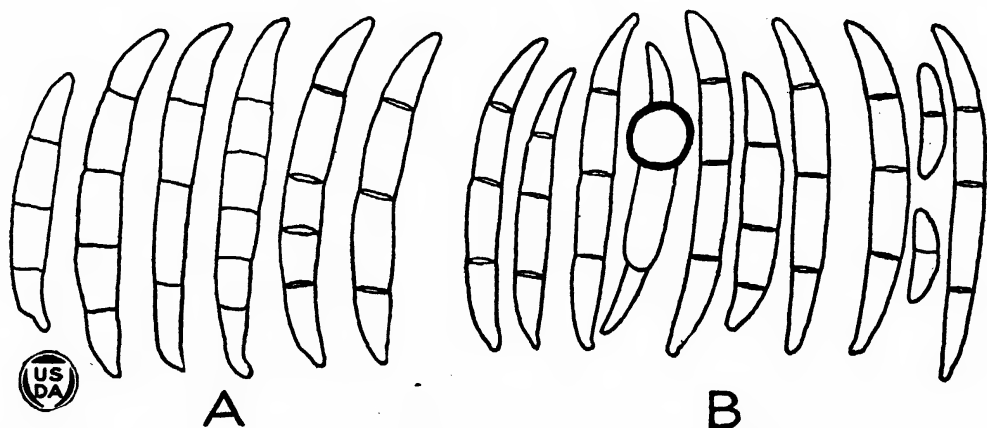


FIG. 1.—Camera lucida outlines of conidia of *Fusarium cepae* A. Sporodochial conidia from 24-day-old culture on red raspberry cane plug. B. Pionnotal conidia from 10-day-old culture on hard lima bean agar

is that the conidia are typically broader toward, more curved near, and sometimes suddenly constricted at the apex. The strain here discussed never showed this last character. It agrees closely with *F. hyperoxysporum* as to size and shape of conidia, but differs decidedly in its general appearance on various media. It differs from *F. oxysporum* in having slightly larger spores and in the total absence of sclerotia. It agrees with Hanzawa's description (4) of *Fusarium cepae* as to septation, curvature, and length of conidia, but differs slightly as to width of conidia and size of chlamydospores. Its measurements of 3-septate conidia average 3 to  $5\mu$ ; he records 4.5 to  $6.3\mu$ . In his figures he has illustrated the swollen conidia; and if we assume that he also measured the maximum width of the swollen conidia, rather than the width at the septa, the discrepancy is probably accounted for. Wollenweber (11, p. 173) mentions the importance of considering this point in comparing the descriptions of *Fusaria*.

#### TEMPERATURE RELATIONS

Petri dishes of equal size containing equivalent amounts of hard potato agar (2 per cent glucose) were inoculated in the center with the organism and placed in duplicate in a series of incubators ranging from 4° to 35° C. Growth occurred over the entire range, but very slowly at the extremes. The diameter of the colonies after five days was taken as a criterion of comparison, the results being represented graphically in Figure 2. There is a gradual increase in the rate of growth up to 25°, with a distinct optimum at about 25° to 28°, and a rapid drop beyond 30° C.

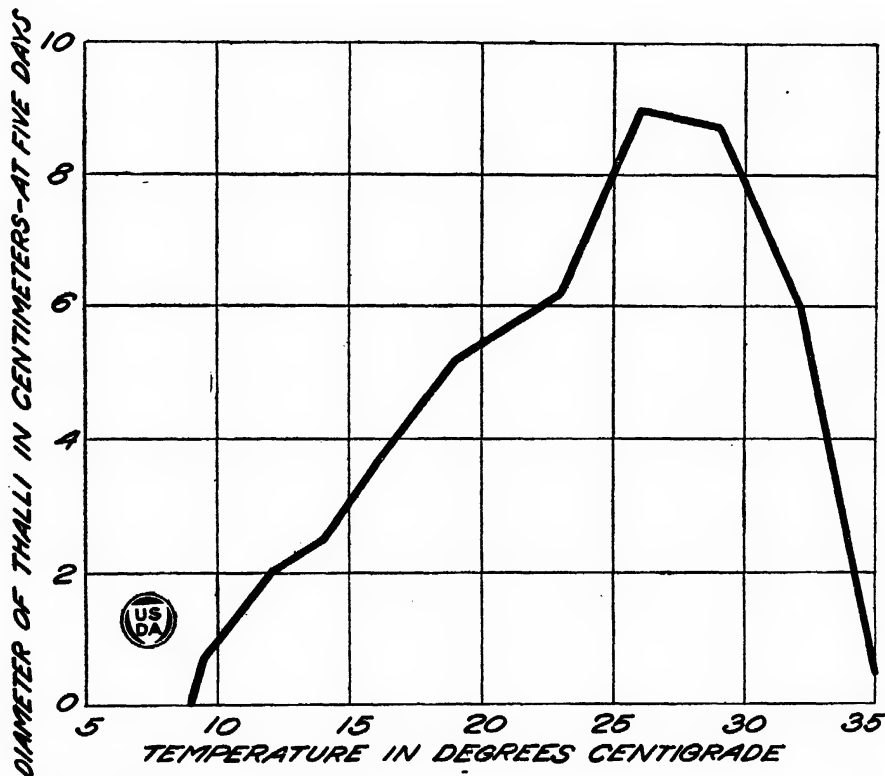


FIG. 2.—Relation of temperature to growth of *Fusarium cepae* on potato agar plates

RELATION OF ACIDITY AND ALKALINITY

In order to study the reaction of the fungus to various concentrations of H and OH ions the methods outlined by Karrer and Webb (5) were followed with slight modifications. Erlenmeyer flasks of 150 cc. capacity containing 50 cc. each of Richard's solution adjusted to various P<sub>H</sub> values were inoculated with equivalent amounts of a spore suspension of the fungus. After 10 days' incubation at 22° C. the mycelial mats were filtered on weighed filter paper, dried at 90° C. and the weight determined. The P<sub>H</sub> values of the solutions at the beginning of the experiment and after removal of the mycelium were determined. These with the dry weights of the mycelium are given in Table I.

TABLE I.—Growth of *Fusarium cepae* in Richard's solution adjusted to various P<sub>H</sub> values after 10 days at 22° C.

P <sub>H</sub> value		Dry weight of mycelium
At beginning of experiment	After removal of mycelial mat	
1.8	1.8	No growth.
2.2	2.2	0.0050
2.8	2.8	.0256
2.8	2.8	.0240
5.0	4.6	.2262
6.6	6.8	.2340
7.4	7.2	.1845
8.2	8.0	.1460
8.4	8.2	.0209
9.2	8.2	No growth.
9.6	8.4	No growth.

The fungus grows best in a slightly acid medium. There was very little change in the reaction of the medium during the experiment, even at the points where most profuse growth occurred. The acidity of the scale tissue of nearly mature onion bulbs of the red, yellow, and white types was found to vary between  $P_H$  5.3 and 5.4. The fungus grows well beyond the acidity limits ordinarily encountered in the host tissue. Moreover one would expect the reaction of the soil to materially affect the fungus only in the case of very high acidity or alkalinity.

#### PATHOGENICITY

The repeated isolation of this species from decaying onion bulbs was evidence of its pathogenic properties. This fact was further substantiated by inoculation of growing onion plants and of mature bulbs.

#### INOCULATIONS IN THE GREENHOUSE

Apparently healthy onion sets were used; they were prepared by removing the dry outer scales, sterilizing in 1 to 1,000 mercuric chlorid solution for 15 minutes, and rinsing several times in sterile water. Each bulb was wounded by piercing the base of the scales with a sterile needle. Those bulbs to be inoculated were placed in a water suspension of macroconidia of the fungus for an hour or longer; those to be used as controls were similarly exposed to sterile water. Subsequently the bulbs were removed and planted in pots of soil in a greenhouse in which the air temperature ranged from 18° to 24° C.

Several experiments following this plan were conducted and the final results are given in Table II. Both inoculated and uninoculated bulbs produced leaves promptly, the top growth of the former, as a rule, being noticeably reduced from the outset. The first signs of disease usually appeared on the tenth to twelfth day. The green color at the tips of the leaves faded somewhat followed by a gradual loss of turgidity. Progressive wilting of the tops through several days resulted finally in complete death of the plant. (See Pl. 2, A.) Simultaneously a decay of the leaf bases below ground occurred. The progress of the disease, as will be shown later, is materially influenced by temperature.

TABLE II.—*Results of greenhouse inoculation experiments with Fusarium cepae*

Experiment number	Number plants inoculated	Plants diseased	Number of controls used	Condition of controls at end of experiment
		<i>Per cent.</i>		
1	18	100	18	Healthy.
2	24	100	24	Do.
3	14	100	14	Do.
4	32	100	24	<sup>a</sup> Do.
5	10	100	8	Do.

<sup>a</sup> One bulb affected with bacterial decay; no evidence of *Fusarium* rot present.

One hundred per cent infection resulted in each inoculation experiment while the controls remained free from *Fusarium* rot. The fungus was repeatedly reisolated from diseased plants and was proved to be identical with the original culture. When wounded bulbs were planted in sterilized soil inoculated with a spore suspension of the organism, the disease developed in a quite similar manner and 80 per cent of the plants became infected.

It is quite evident that the fungus is an aggressive wound parasite of onion plants. In order to determine whether or not the fungus is capable of infecting through the unbroken surface, 18 apparently sound sets were planted in inoculated soil. The same number of bulbs were planted in sterilized soil as controls. In four of the plants in inoculated soil the characteristic symptoms of the disease developed and from them the fungus was reisolated. The other plants remained healthy, as did the controls. It thus appears that the fungus will invade apparently sound plants but the percentage of infection is much less than in the case of wounded plants. It is well to recall here the common observation that

this *Fusarium* bulb rot is associated with onion maggot injury. It is obvious from the experimental evidence just given that mechanical injury to the base of onion bulbs due to insects or other causes will greatly facilitate infection by *Fusarium cepae*.

#### INOCULATIONS IN THE FIELD

Inoculation experiments were continued out of doors at Madison, Wis., in 1922 on soil which had never previously grown onions. The soil was inoculated at the time of planting (April 22) by the addition of a spore suspension of the organism. Plats of uninoculated soil were planted as controls. Bulbs were grown from bottom sets of White Portugal, Red Wethersfield, and Yellow Strassburg varieties, and from seed of Crystal Wax Bermuda, Crimson Globe, Silver Globe, Golden Globe, and White Portugal varieties of onion. Plantings from seed of White Welsh onion (*Allium fistulosum*) and leek (*Allium porrum*) were included. The development of the disease followed closely that observed previously under natural field conditions. Approximately equal numbers of plants from inoculated and uninoculated plats were examined on August 8. The results are given in Table III. It is significant to note that infection took place in the case of every variety of common onion (*Allium cepae*) tried. Varieties representing each of the three color types (white, yellow, and red) were included and no marked differences in varietal susceptibility were noted. The Welsh onion and leek are apparently decidedly resistant.

TABLE III.—Results of field inoculations with *Fusarium cepae* upon different varieties of common onion and upon Welsh onion and leek

Method of propagation	Variety	Number of bulbs examined at end of season	Per cent showing <i>Fusarium</i> bulb rot	Condition of controls
From sets....	Red Wethersfield, White Portugal, Yellow Strassburg..	215	22	Healthy.
	Red Wethersfield.....	150	15	Do.
	Crystal Wax Bermuda.....	300	<sup>a</sup> 2	( <sup>a</sup> )
	Red Globe.....	175	12	Healthy.
	White Globe.....	295	18	Do.
From seed....	Yellow Globe.....	75	8	Do.
	White Portugal.....	300	7.5	( <sup>b</sup> )
	White Welsh.....	400	0	Healthy.
	Leek.....	400	0	Do.

<sup>a</sup> No *Fusarium* rot in checks; results were obscured by the abundance of soft rot in both inoculated plants and checks following early maturity of this variety.

<sup>b</sup> One bulb was diseased and *Fusarium* isolated therefrom.

#### INOCULATIONS IN STORAGE

Although the fungus was found constantly associated with the decay of onion bulbs, the first attempts to reproduce the disease by artificial inoculation of mature bulbs were unsuccessful. Insertion of spores into wounds at the base of the bulbs did not result in infection under ordinary storage conditions, although the disease was known to progress in naturally infected bulbs in the same environment. Holding of the bulbs continuously at a high relative humidity was undesirable because this condition brought about almost immediate sprouting. It was found, however, that the typical bulb rot could be produced if the bulbs were inoculated as indicated above and held for 48 hours in the saturated atmosphere of moist chambers at room temperature and then placed in wire baskets under ordinary laboratory conditions. Thirty onion sets thus inoculated and incubated at 22° C. were completely decayed after 30 days while the same number of controls showed only slight desiccation and remained perfectly healthy. The experiment was successfully repeated with large bulbs of both the white and red types. In the case of the small sets the decayed tissues dried out rapidly and a "dry rot" was produced. With the larger bulbs the greater amount of succulent tissue yielded a somewhat more "watery decay" and the greater amount of moisture brought about the production of more aerial mycelium which was most pronounced as a white fluffy mass at the base of the bulb. (See Pl. 2, B.)

## RELATION OF SOIL TEMPERATURE AND MOISTURE TO THE DISEASE

The importance of studying the relation of environmental conditions to the occurrence of this disease is obvious. Since the organism is a soil fungus and infection occurs normally in the subterranean parts of the plant, soil temperature and soil moisture were considered first.

Temperature studies were carried out in Wisconsin soil temperature tanks. Inoculations were made, as previously described, by first placing the wounded bulbs in a spore suspension and then planting in metal cans of soil, four bulbs to a can. The soil moisture was adjusted to 80 per cent of the water-holding capacity, and the cans were then placed in the constant temperature tanks held at 30°, 26°, 22°, 19°, 15°, and 12° C. An equal number of uninoculated controls were included in each case. The air temperature ranged from 15° to 20° and was uniform for all tanks.

No signs of the disease developed at 12° C. even after several weeks. All of the inoculated plants held at 15° to 30° eventually succumbed to the typical wilt of the tops and decay of the bulbs, but a decided difference prevailed in the progress of the disease at the various temperatures. The bulbs all sent up tops very quickly. The first signs of the wilt were noted on the tenth day at 26° and at 30° and the disease progressed very rapidly at these temperatures, resulting in entire collapse of the plants by the twentieth day. The controls at these temperatures had reached a height of eight inches at this time. The plants at 22° and at 19° were proportionately slower in succumbing to wilt. At 15° there was no sign of wilt until the twenty-fourth day and the last of the four plants was not affected until the thirty-sixth day. The experiment when repeated and extended to 32° gave confirming results and the disease was nearly as active at 32° as at 28°. (See Pl. 3.) The controls all remained healthy with the exception of one plant at 30° which wilted slightly.

The results show the fungus capable of producing the disease at a range of considerable width extending from 15° to 32° C. or above. Within these limits there is an optimum at about 28° and a gradual retardation of progress as the temperature lowers. The disease, as affecting the growing plant, is apparently inhibited at 12°.

Constant soil moistures are not so readily obtained. To avoid as much as possible variations in the can due to force of gravity upon the soil water, shallow metal pots were chosen (6 inches deep and 5 inches in diameter). Sterilized greenhouse clay loam soil was adjusted in various lots to 100 per cent, 85 per cent, 50 per cent, and 26 per cent of its water-holding capacity, as determined by the standard 10-inch cylinder. The soil was then potted, care being taken to so pack it that equivalent amounts by dry weight occupied equivalent volumes throughout the series. In the case of the highest moisture, final adjustment was made after the soil was potted. Constant moisture content was approximated by daily weighing and adjustment. At the end of the experiment moisture determinations of samples from the upper 2 inches of each of the pots were made.

In pots of soil thus prepared, bulbs wounded and inoculated in the usual manner were planted, 4 bulbs to each pot. These pots were placed in a greenhouse held at 20° to 25° C. Although the range of soil used extended from a very dry soil to one practically saturated, little difference was noted in the rate at which the disease developed. (See Table IV.) It appears that soil moisture within the range at which the onion plant will normally develop, does not materially affect the *Fusarium* wilt or decay after infection has once occurred.

TABLE IV.—Relation of soil moisture to development of *Fusarium cepae*

Original moisture content (percentage of dry weight)	Moisture content of surface 2 inches at end of experiment	Approximate per cent of water-holding capacity	Number of plants inoculated	Days elapsing before all plants had wilted	Number of control plants	Number of checks wilting
10.5	9.5	26	12	14	4	0
21	25	50	<sup>a</sup> 12	20	4	0
31	33	85	12	14	4	0 <sup>b</sup>
37	37.5	100	12	15	4	0 <sup>b</sup>

<sup>a</sup> One plant had not wilted at end of experiment.  
<sup>b</sup> One bulb rotted completely without sprouting.

The next experiment consisted essentially of a repetition of the soil temperature studies with the exception that two complete series were included—one in soil adjusted to 30 per cent and the other to 75 per cent of its water-holding capacity. The results given in Table V show again the influence of temperature on the development of the disease.

The plants in the high moisture series sprouted and grew much more rapidly than those in the lower moisture cans, and attained a much greater final growth. The same was true with the check plants in the high-temperature tanks, as compared with the lower ones, but the greatest final growth took place in the high-moisture cans in the 19° C. tank. The first evidence of the effect of the fungus on the plants appeared simultaneously in the high and low moisture cans in the 26° tank on the eighth day. A day later the inoculated plants in the 30° and 26° tanks began to wilt, followed two days later by one in the 19° tank. Although the wilt appeared in the 19° and 22° tanks shortly after it appeared at the higher temperatures, its progress was much slower in the former. This agrees with former temperature experiments. At 12° the disease did not appear in either lot after 35 days, showing this to be below the minimum temperature for its development. The results again show that there is no appreciable difference in the rapidity of *Fusarium* rot under widely different soil-moisture conditions. It is to be noted, however, that in all these experiments the fungus was injected into the tissue of the bulb at the beginning. Therefore the results should not be interpreted as necessarily indicating the effect of soil moisture upon initial infection but rather upon the progress of the disease after infection.

TABLE V.—Effect of soil-moisture content upon the progress of *Fusarium cepae* at various soil temperatures

Temperature	Days elapsing before first plant wilted		Days elapsing before all plants wilted		Condition of controls
	Thirty per cent water-holding capacity <sup>c</sup>	Seventy-five per cent water-holding capacity <sup>d</sup>	Thirty per cent water-holding capacity <sup>c</sup>	Seventy-five per cent water-holding capacity <sup>d</sup>	
° C.					
30	9	9	11	12	Healthy.
26	8	8	10	10	Do.
22	9	9	17	16	Do.
19	11	11	19	19	Do.
12	( <sup>a</sup> )	( <sup>a</sup> )	( <sup>a</sup> )	( <sup>a</sup> )	( <sup>b</sup> )

<sup>a</sup> None of the plants showed signs of wilt at 45 days.  
<sup>b</sup> One plant attacked by *Aspergillus* and *Penicillium* but inoculated plants not affected.  
<sup>c</sup> 11.1 per cent of the dry weight.  
<sup>d</sup> 27.8 per cent of the dry weight.

## RELATION OF TEMPERATURE TO OCCURRENCE AND DISTRIBUTION

The final interpretation of field observations in the light of these experimental data must be delayed until a thorough survey of *Fusaria* associated with bulb rot and a study of their morphological and physiological characters has been completed. However, it is significant to note that at least in the Middle West, where this disease is now common, it does not ordinarily appear until July or later. An examination of soil-temperature records (see fig. 3) taken at 1-inch depth in an onion field at Racine, Wis., on two extreme seasons, 1915 and 1916, shows that in this rather northerly section the daily mean does not ordinarily reach 15° C., the lower limit for this disease, for any considerable period until May 15 or later, while the optimum temperature is not reached until July 1 or later. It would therefore seem plausible to believe that soil temperature limits the progress of the disease, so far as this species of *Fusarium* is concerned, during the early half of the growing period.

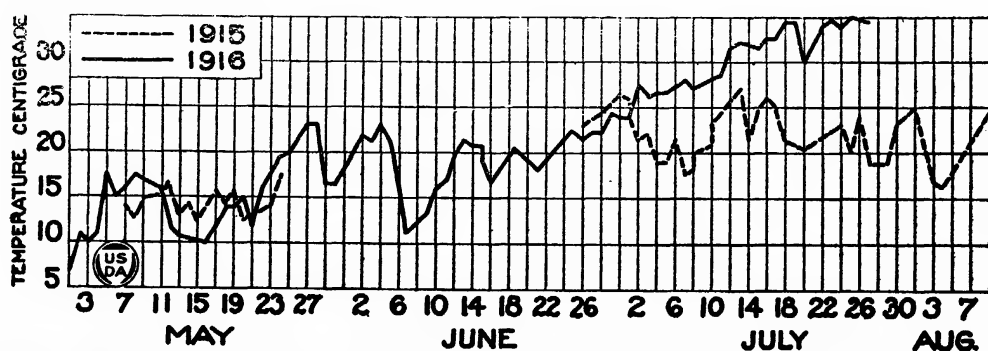


FIG. 3.—Chart from data collected at Racine, Wis., during 1915 and 1916, showing the daily mean soil temperature at a depth of 1 to 2 inches

The distribution of the *Fusarium* bulb rot is not sufficiently well known to interpret any limits on the basis of soil factors. It is significant, however, to note that it has appeared perhaps in its most severe form in the Walla Walla section of Washington and in the Uncompahgre section of Colorado, both of which regions are subject to very high temperatures during the last part of the onion-growing season. Moreover in a recent survey of onion diseases in Europe, the senior writer found *Fusarium* bulb rot doing severe damage only in the irrigated section at Valencia, Spain. Here again the disease appears in the hot midsummer and the general conditions of soil and air environment are very similar to those of the Walla Walla section. A repetition of the soil temperature experiments described above with two pathogenic strains of *Fusarium* collected in the Valencian section showed that these forms coincide very closely with *Fusarium cepae* in their temperature reaction.

## RELATION OF STORAGE TEMPERATURE TO THE DISEASE

A thorough study of storage conditions as affecting the progress of a bulb rot should include a study of humidity as well as temperature. Since facilities for controlling humidity were not available, some preliminary experiments were conducted in which only the temperature was varied.

Experiment 1: Sixty small white onion sets were prepared for inoculation in the usual manner and divided into two equal groups. One group remained without further treatment to serve as controls while the other 30 bulbs were inoculated by insertion of macroconidia of the organism into the wound. The two groups were next placed in separate moist chambers at 22° C. for 48 hours and were



then subdivided into three equal lots as follows: Lot 1, consisting of 10 inoculated and 10 uninoculated bulbs, was placed in wire baskets in an incubator held at 21° to 22° C.; lot 2 was held at 13° to 18°; and lot 3 was held at 5° to 14°. The inoculated bulbs in lot 1, when examined 30 days later, were completely decayed, the succulent tissue having almost disappeared, leaving the outer dry scales in their original position. In lot 2 the decay was nearly as complete, desiccation not having progressed quite so far. At 5° to 14° the decay extended only through the immediate center of the bulbs. The controls remained healthy in all cases.

Experiment 2: One hundred and sixty white sets ( $\frac{3}{4}$  to  $1\frac{1}{2}$  inches in diameter) were divided into four lots of 20 inoculated and 20 uninoculated bulbs each. They were placed at 32°, 20° to 21°, 11° to 16°, and 8° C., respectively. After 19 days the inoculated bulbs at 32° were completely decayed and desiccated; at 20° to 21° the rot was well advanced but the tissue was more watery than at 32°; at 11° to 16° the inoculated bulbs were only slightly decayed but were sprouting profusely; while at 8° the decay was very slight. After 37 days the inoculated bulbs at 11° to 16° had all rotted, with two exceptions, after having produced sprouts of an average length of 5 inches. After 62 days the inoculated bulbs at 8° showed slight decay, usually only adjacent to the wound. The striking effect of inoculation upon this lot was the stimulation of premature sprouting in spite of very slight decay. All the inoculated bulbs had sprouts 2 to 6 inches long while of the controls only four had short sprouts, although they were wounded in the same way as the inoculated bulbs. The controls in all the lots remained healthy without exception.

These experiments indicate that at the higher temperatures (30° C.) there is a very rapid "dry rot" of the onion bulb when inoculated through a basal wound. The desiccated condition of the tissue is probably brought about by the increased evaporation due to high temperature and somewhat lower relative humidity. As the temperature is reduced the progress of the rot is slower until at 8° there is very slight decay but excessive sprouting. The latter is an important factor in onion storage, since sprouting makes the bulb worthless for market purposes, and thus *Fusarium* infection may be indirectly responsible for considerable losses on onions held at low temperatures. It is also of interest to note that the fungus is active at a lower temperature in the case of the dormant bulb than in that of the growing plant.

### SUMMARY

*Fusarium* bulb rot of onion is a disease of increasing importance in the United States. A comprehensive survey of *Fusaria* associated with the disease in various parts of the world has been started by Dr. G. K. K. Link. The present paper is a study of the disease as caused by a single strain which appears to be the chief causal agent of the disease in the Middle West. A description of the disease under mid-western conditions is given.

The organism, *Fusarium cepae* Hanzawa, belongs to the section *Elegans* and is very close to *Fusarium hyperoxysporum* Woll., *F. redolens* Woll., and *F. oxysporum* Schlecht. Its morphological and cultural characters are recorded.

On potato agar the fungus grows over a range of from 4° to 35° C. Optimum growth takes place between 25° and 28°.

On Richard's solution the fungus grows over a range in  $P_H$  from 2.2 to 8.4; best growth occurs at  $P_H$  6.6. The fungus thus grows well within that range of acidity or alkalinity ordinarily encountered in the host tissue or in the soil.

Inoculations were readily secured through wounds in plants growing from sets in the greenhouse. A much smaller percentage of infections was secured

when the plants were not wounded. As high as 22 per cent infection was secured in plants grown out of doors, from sets and from seed, on previously inoculated soil. Bulbs inoculated through wounds and kept under ordinary laboratory conditions very seldom became infected. Exposure in a moist chamber for 48 hours after inoculation followed by removal to laboratory conditions resulted usually in 100 per cent infection.

In controlled soil-temperature experiments the disease developed within the limits of 15° to 32° C. Most rapid development occurred at 28° to 32°. Progress was much slower as the temperature decreased. The disease did not develop at 12°.

Variation in soil moisture had little or no effect upon the progress of the disease after initial infection had taken place.

The results of soil-temperature experiments coincide with the occurrence of the disease in the field. In southeastern Wisconsin the soil temperature mean at 1 to 2 inches does not ordinarily reach 15° C. until May 15 or later while the optimum temperature for the disease is not reached until July 1 or later. The disease in this section does not appear until after the latter date. The most serious outbreaks of *Fusarium* bulb rot in the United States are in the Walla Walla section of Washington and the Uncompahgre section of Colorado; in Europe, the Valencian district of Spain suffers most. All three sections are subject to extremely hot weather during the latter part of the onion-growing season.

As a storage disease the bulb rot is most active at or above room temperature. At about 30° C. the tissues decay and desiccate rapidly. At 20° the decay is rapid but the tissue remains watery for a longer time. At about 15° the decay is very slow but premature sprouting is evident. At around 8° the rot is very slight indeed, but the promotion of premature sprouting in inoculated bulbs is very marked. Thus at this low temperature the indirect effect of the disease may cause heavy losses in storage or transit.

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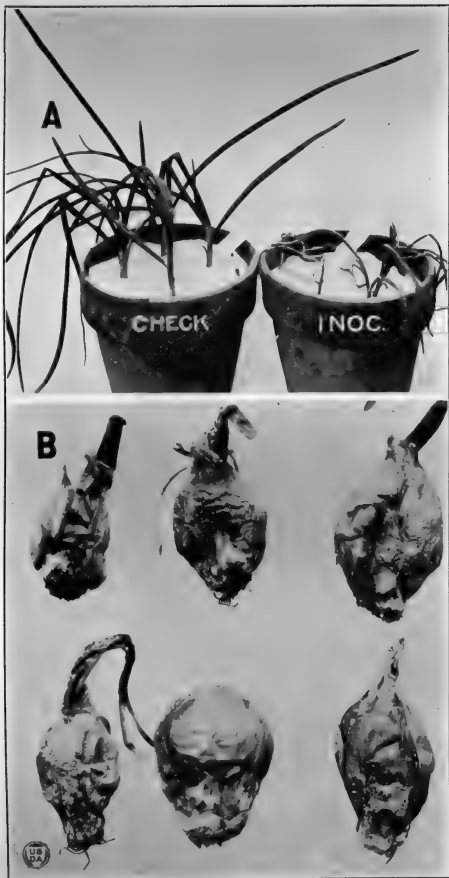
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## PLATE 1

White variety of onions showing infection by the bulb-rot *Fusarium* as it occurs on the immature plants three or four weeks before harvest. Note the yellowing and loss of turgidity beginning at the tips of the leaves and the decay of the scales and root system starting at the stem plate. Specimens collected at South Holland, Ill., July, 1918.





## PLATE 2

Inoculation experiments with *Fusarium cepae*.

A.—White Portugal onion sets were inoculated with spores through needle punctures at the base of the bulbs, and planted in sterilized soil in the greenhouse at about 22° C. Checks were wounded but not inoculated. Both lots produced top growth promptly, but the inoculated plants grew less rapidly. The disease appeared as loss of turgor and yellowing at the tips of the leaves on the tenth day. Progressive yellowing and wilting continued, while the checks grew as usual. Photographed 20 days after inoculation.

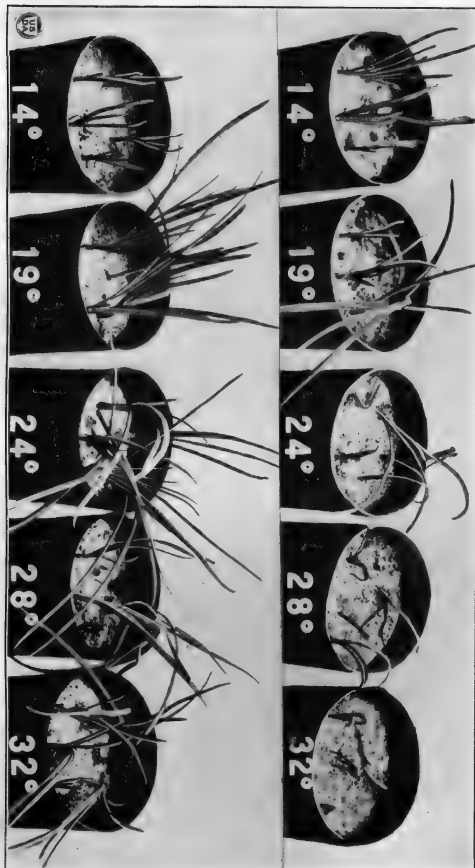
B.—Large bulbs, Red Globe variety, inoculated with spores through needle punctures at base, placed in moist chambers for 48 hours, and then removed to laboratory temperature and humidity. Typical decay and shrinkage of tissue resulted with conspicuous production of mycelium on the exterior of outer rotted scales. Photographed 23 days after inoculation.

### PLATE 3

Relation of soil temperature to the progress of *Fusarium cepae*.

Upper row: Plants from White Portugal sets inoculated and planted as described for Plate 2, A, and held at various soil temperatures. Lower row: Plants from uninoculated bulbs held at the same temperatures. Photographed 18 days after initiation of the experiment. The disease has not developed at 19° C. nor at 14° C. At 24° the plants have recently wilted. At 28° and 32° the plants were killed rapidly before the tops had grown to any great extent.





# THE CHLORID CONTENT OF THE LEAF TISSUE FLUIDS OF EGYPTIAN AND UPLAND COTTON<sup>1</sup>

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## INTRODUCTION

In earlier investigations of the physico-chemical properties of the leaf tissue fluids of Pima Egyptian and Acala and Meade Upland cotton (5)<sup>3</sup> we have shown that when grown under irrigation at the United States field station in the Gila River Valley at Sacaton, Ariz., the Egyptian cotton is characterized by higher osmotic concentration, as measured in terms of freezing point depression,  $\Delta$ , and higher specific electrical conductivity,  $\kappa$ , of its tissue fluids than either of the two Upland varieties.

The consistently higher osmotic concentration of the tissue fluids of the Egyptian cotton might be due to either greater capacity for the synthesis of organic solutes or to greater capacity for the absorption and for the retention in solution of electrolytes from the soil. The higher constants for the specific electrical conductivity,  $\kappa$ , of the tissue fluids of the Egyptian cotton point to the absorption and tolerance in solution of electrolytes as important factors in the differentiation of the physico-chemical properties of the tissue fluids of the two types of cotton.

In analyzing the physiological differences between the Egyptian and Upland types more minutely, therefore, it becomes of importance to determine the concentration of specific solutes in the tissue fluids.<sup>4</sup>

The desirability of an investigation of the chlorid content of the tissue fluids of the Egyptian and Upland types of cotton is suggested by the early observations of Kearney and Means (7) and of Kearney (6) on the growth of cotton in saline soils. Furthermore, Balls (1, 2) has called attention to the relatively high chlorid content of cotton plants grown in Egypt.

In view of these observations, and of the differences in the physico-chemical properties of the tissue fluid of Pima Egyptian, Meade, and Acala Upland cotton already distinguished (5), the determination of the concentration of chlorids in the tissue fluids of different varieties might seem to be of particular importance in selecting crop plants which may best be grown in regions of so-called alkali soils, in many of which the content of chlorids is very high.

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<sup>1</sup>Received for publication February 16, 1924.

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<sup>3</sup>Reference is made by number (italic) to "Literature cited," p. 704.

<sup>4</sup>This has been one of the primary purposes of this phase of the work carried out on cotton by the Office of Alkali and Drought Resistant Plant Investigations, the Office of Biophysical Investigations, and the Office of Western Irrigation Agriculture, of the Bureau of Plant Industry, United States Department of Agriculture, since 1921. For the opportunity of joining in this program we are indebted to T. H. Kearney, G. N. Collins, and C. S. Scofield, in charge of the three offices mentioned.

## MATERIALS AND METHODS

The data are derived from four experimental series of plants grown at the United States field station, Sacaton, Ariz. These may be numbered for convenience of reference as follows:

A.—A comparison of Pima Egyptian and Acala Upland cotton in 1920.

B.—A comparison of Pima Egyptian and Meade and Acala Upland cotton in 1922.

C.—A second comparison of Pima Egyptian and Meade Upland cotton in 1922.

D.—A comparison of Pima Egyptian and Lone Star Upland cotton in 1923.

The two or three varieties of plants to be compared were distributed uniformly over the plots available in order to avoid the influence of substratum heterogeneity (3, 4). In this regard the technique was essentially that of the experiments made in 1921 (5).<sup>5</sup>

The first comparison of Pima Egyptian and Meade and Acala Upland cotton in 1922 (Series B, above) was made by planting 24 rows each 180 feet in length and consisting of six cultures of 10 feet each of Acala, Pima, and Meade.<sup>6</sup>

In the collection of samples, the field was in effect divided into 216 subplots (each 10 feet long and two rows wide, and planted to one of the three varieties) by combining rows 1 and 2, 3 and 4, etc. The samples of tissue were drawn as widely and as uniformly as possible from the theoretically 20<sup>7</sup> plants of each subplot. The three adjoining subplots of the same pair of rows serve as units in the making of comparisons. Each unit therefore comprises determinations on Pima, Acala, and Meade plants. The samples of each unit were taken simultaneously, so that external conditions which vary with time can not influence the results.

This comparison (B) involves a first and a second series of determinations, the first based on samples collected from July 25 to August 9, the second based on samples collected from the same cultures between August 28 and September 4, inclusive.

The second comparison (C) of Pima Egyptian and Meade Upland cotton made in 1922 was based on samples of tissue collected from individual plants of the two varieties. These plants were distributed uniformly over a plot 26.5 by 400 feet in area. They were interspersed among plantings of five newly imported Egyptian varieties which do not require consideration here. The Pima and Meade plants were separated by only three hills of F<sub>2</sub> hybrid plants. Tissue samples were collected from August 21 to August 27, inclusive.

The comparison of Pima Egyptian and Lone Star Upland cotton in 1923 was made on an irrigation plot of the standard dimension, which was selected because of the high salt content of the soil at the north end. The south half of the plot had lain in alfalfa from 1920 to 1923. The north half was too saline to permit a growth of alfalfa, and but few cotton plants survived on this portion of the plot. The collections made from the plants of the 10-foot subrows at the south and the north half of the plot will, therefore, be discussed separately.

<sup>5</sup> This statement applies to the cultures made in 1922 and 1923. The few determinations made in 1920 were secured incidental to other determinations.

<sup>6</sup> The 24 rows were distributed over the north half of three irrigation plots, each 26.5 by 400 feet in area. Ten feet at each end were used for buffer plants in order that the end plots used might have the same cultural conditions as those in the center of the field.

<sup>7</sup> Because of the cold spring, germination was poor and many plants were wanting. The number was still further reduced by the development of root rot late in the season.

Samples of tissue were collected and the fluids extracted, and a number of physico-chemical constants determined<sup>8</sup> in essentially the manner described in an earlier paper (5). The chlorid content was determined by precipitating the chlorids as silver chlorid by the addition of a known amount of standard silver nitrate solution and modifying all organic substances by digesting on a sand bath with boiling nitric acid. The excess silver nitrate was then titrated with potassium thiocyanate, with iron alum as indicator.

The samples for the series of 1920 and 1922 were preserved in sealed tubes and analyzed later. In all these the precipitated silver chlorid was removed by filtration. The analyses for 1923 were made on the fresh tissue fluids. In these determinations titration was made in the presence of the precipitated silver chlorid, as has been shown to be allowable (8) in this method.

All results are expressed as chlorids in terms of grams of Cl per liter of tissue fluids. Duplicate analyses were made in a rather large proportion of the cases. The two determinations have been averaged to obtain the constant to be used to represent the plants unless there was some evidence that one of the two analyses was more accurate than the other. When one sample contained 10 cc. whereas the other contained less than 5 cc. the constant determined from the larger sample has generally been taken.<sup>9</sup>

### PRESENTATION OF RESULTS

Only one pair of determinations of chlorid content are available for cotton grown in 1920. These seem to indicate a higher concentration of Cl in the Egyptian than in the Upland types.

TABLE I.—*Chlorid content of the leaf tissue fluids of Egyptian and Upland cotton grown in 1920*

Variety of cotton	Number of sample <sup>a</sup>	Gm. of chlorids (Cl) per liter
Pima Egyptian	(1)	6.29
Acala Upland	(2)	4.43
	(1) — (2)	+ 1.86

<sup>a</sup> The sample numbers are the same as those used to designate the determinations given elsewhere (5).

In each experiment except that for 1920, relatively large numbers of analyses were made on samples so taken that each determination based on tissues collected from Egyptian plants was provided with a control sample taken from Upland plants grown under as nearly as possible the same conditions.

The comparisons for these large series may be most crucially made by means of statistical constants and their probable errors. The differences between the varieties compared are, however, sufficiently large that they may be brought out by means of a mere tabulation of the analyses.

<sup>8</sup> These will be considered elsewhere in relation to another group of problems.

<sup>9</sup> We have to thank Supt. C. J. King for many courtesies which facilitated our work at Sacaton. George J. Harrison was responsible for the making of the rather complicated plantings and for the superintendence of the cultures throughout the season. We are greatly indebted to G. O. Burr, Charles W. Crane, R. D. Evans, A. H. Johnson, W. B. Sinclair, and A. T. Valentine, field assistants in 1922 and 1923, for their large part in the collection and preparation of the samples for analysis.

The data for the larger series of determinations on Pima Egyptian and Meade and Acala Upland cotton made in 1922 (comparison B, above) and for theser ies of determinations made on Pima Egyptian and Lone Star Upland cotton in 1923 <sup>10</sup> (comparison D, above) appear in Table II. For convenience of tabulation the chlorid contents may be grouped in classes of 0.25 gm. of Cl per liter range.

TABLE II.—Frequency distribution of chlorid content (in terms of grams of Cl per liter of fluid) in leaf tissue fluids of Egyptian and Upland cotton as grown at Sacaton, Ariz., in 1922 and 1923

Grams of chlorid (in terms of Cl) per liter		Cultures in 1922						Cultures in 1923					
		First series, July 25 to Aug. 9			Second series, Aug. 28 to Sept. 4			First series, July 29 to Aug. 14		Second series, Part I, Aug. 18 to Aug. 31		Second series, Part II, Aug. 24 to Aug. 30	
		Pima	Acala	Meade	Pima	Acala	Meade	Pima	Lone star	Pima	Lone star	Pima	Lone star
0. 126-0. 375	0. 250	-----	-----	-----	-----	-----	-----	-----	1	-----	-----	-----	-----
0. 376-0. 625	0. 500	-----	-----	-----	-----	-----	-----	-----	1	-----	2	-----	-----
0. 626-0. 875	0. 750	-----	-----	-----	-----	-----	-----	-----	18	-----	15	-----	1
0. 876-1. 125	1. 000	-----	-----	-----	-----	-----	-----	-----	23	-----	21	-----	1
1. 126-1. 375	1. 250	-----	-----	-----	-----	-----	-----	-----	24	-----	16	-----	2
1. 376-1. 625	1. 500	-----	-----	1	-----	-----	2	2	2	-----	12	-----	2
1. 626-1. 875	1. 750	-----	-----	-----	-----	-----	3	7	1	2	1	-----	1
1. 876-2. 125	2. 000	-----	-----	1	-----	-----	6	11	-----	-----	1	-----	-----
2. 126-2. 375	2. 250	-----	-----	5	-----	2	6	16	1	1	1	1	2
2. 376-2. 625	2. 500	-----	-----	10	-----	-----	12	10	-----	9	1	2	1
2. 626-2. 875	2. 750	-----	4	12	1	10	10	12	-----	4	-----	-----	2
2. 876-3. 125	3. 000	-----	5	7	-----	11	7	6	-----	10	-----	-----	-----
3. 126-3. 375	3. 250	1	13	8	-----	12	9	2	-----	14	-----	2	1
3. 376-3. 625	3. 500	3	16	11	1	13	8	3	-----	12	-----	-----	-----
3. 626-3. 875	3. 750	6	11	2	2	7	3	1	-----	9	-----	2	-----
3. 876-4. 125	4. 000	5	8	6	2	1	3	-----	-----	4	-----	2	-----
4. 126-4. 375	4. 250	12	6	2	3	7	-----	-----	-----	3	-----	2	-----
4. 376-4. 625	4. 500	5	3	3	9	5	-----	-----	-----	-----	-----	-----	-----
4. 626-4. 875	4. 750	11	3	-----	5	1	-----	-----	-----	3	-----	1	-----
4. 876-5. 125	5. 000	8	1	-----	6	1	-----	-----	-----	-----	-----	1	-----
5. 126-5. 375	5. 250	3	-----	-----	8	-----	-----	-----	-----	-----	-----	-----	-----
5. 376-5. 625	5. 500	4	1	1	12	-----	-----	-----	-----	-----	-----	-----	-----
5. 626-5. 875	5. 750	4	-----	-----	7	-----	-----	-----	-----	-----	-----	-----	-----
5. 876-6. 125	6. 000	2	-----	-----	5	-----	-----	-----	-----	-----	-----	1	-----
6. 126-6. 375	6. 250	4	-----	-----	2	-----	-----	-----	-----	-----	-----	-----	-----
6. 376-6. 625	6. 500	1	-----	-----	2	-----	-----	-----	-----	-----	-----	-----	-----
6. 626-6. 875	6. 750	-----	-----	-----	3	-----	-----	-----	-----	-----	-----	-----	-----
6. 876-7. 125	7. 000	-----	-----	-----	1	-----	-----	-----	-----	-----	-----	-----	-----
Totals	-----	68	71	69	69	70	69	70	71	71	70	14	13

<sup>10</sup> The first series of determinations (July 29 to Aug. 14) for the culture of Pima Egyptian Lone Star and Upland cotton made in 1923 was drawn from the south half of the plot only. This was done because the few plants which developed in the north half were so small at this time that it was evident that two collections of leaves could not be made. It seemed desirable to allow them to stand until the second collection of samples was made. The second series of determinations (Aug. 18 to Aug. 31) is divided into two parts. Part I represents collections from the south half of the plot. It is exactly comparable with the first series. Part II comprises the few determinations which it was possible to make (Aug. 24 to Aug. 30) on the scattering plants in the north half of this plot. They will not be discussed in this place farther than to note that the distribution of chlorid content in the two types of cotton is in excellent agreement with that found in the main part (Part I) of the experiment.

**TABLE III.**—*Frequency distribution of chlorid content (in terms of grams of Cl per liter of fluid) in leaf tissue fluids of Egyptian and Upland cotton as grown at Sacaton, Ariz., in 1922*

Grams of chlorid (in terms of Cl) per liter		Frequencies for samples taken Aug. 21 to Aug. 27		Grams of chlorid (in terms of Cl) per liter		Frequencies for samples taken Aug. 21 to Aug. 27	
Class range.	Class center	Pima	Meade	Class range.	Class center	Pima	Meade
2. 626—2. 975	2. 80	-----	4	7. 526—7. 875	7. 70	9	1
2. 976—3. 325	3. 15	-----	7	7. 876—8. 225	8. 05	4	-----
3. 326—3. 675	3. 50	-----	7	8. 226—8. 575	8. 40	7	-----
3. 676—4. 025	3. 85	-----	9	8. 576—8. 925	8. 75	1	-----
4. 026—4. 375	4. 20	-----	3	8. 926—9. 275	9. 10	2	-----
4. 376—4. 725	4. 55	-----	2	9. 276—9. 625	9. 45	1	-----
4. 726—5. 075	4. 90	1	3	9. 626—9. 975	9. 80	2	-----
5. 076—5. 425	5. 25	2	5	9. 976—10. 325	10. 15	3	-----
5. 426—5. 775	5. 60	1	4	10. 326—10. 675	10. 50	-----	-----
5. 776—6. 125	5. 95	1	2	10. 676—11. 025	10. 85	-----	-----
6. 126—6. 475	6. 30	-----	2	11. 026—11. 375	11. 20	1	-----
6. 476—6. 825	6. 65	3	-----	Totals.....	-----	48	49
6. 826—7. 175	7. 00	7	-----				
7. 176—7. 525	7. 35	3	-----				

Because of a higher and more variable chlorid content in the smaller comparison between Pima and Meade Upland cotton made in 1922 (comparison C, above) a wider class value must be used for tabulation. A class range of 0.35 gm. of Cl per liter of fluid was therefore adopted. (See Table III.)

The range of chlorid content in each class and the central values to be used for purposes of calculation are indicated in the first two columns (Tables II and III.) The frequencies show the numbers of analyses falling in each of the classes in the comparisons of Pima Egyptian and Meade and Acala Upland cotton grown in 1922 and in the comparison of Pima Egyptian and Lone Star Upland cotton in 1923.<sup>11</sup>

While these distributions show clearly that the chlorid content of the Upland varieties (Acala, Meade, and Lone Star) is lower than that of the Egyptian variety (Pima) with which they are compared, the chlorid contents vary considerably from sample to sample. The frequency distributions are transgressive, many of the samples of Upland tissue fluids of higher chlorid content exceeding the samples of lower chlorid content in the Egyptian cotton in the concentration of this ion. It might, therefore, be assumed that the difference between the analyses is after all a statistical one, holding in the long run but not demonstrable in individual comparisons. An examination of the analyses for the associated plants of the two types of cotton shows that this is not the case.

The relationship between the individual determinations may be best brought out by means of arrangement in correlation tables. Such tables have been prepared for each of the series, but because of their essential agreement only one (Table IV) will be published.

<sup>11</sup> The frequencies given in these tables represent all the available determinations for each of the varieties. In some cases determinations for one or more of the varieties of a subplot could not be made; hence the totals of these distributions differ among themselves and from those given in the table of differences below (Table V) in which only determinations based on plants which occurred in actual association are given.

The comparison between the Pima Egyptian and Meade Upland cotton as grown in a second series of cultures in 1922 (comparison C, above) appears in Table IV. Because of the higher chlorid content and the greater variability<sup>12</sup> of chlorid content in this experiment the tabulation has been made in units of 0.35 gm. per liter range.

In this arrangement of the data the chlorid content of Upland cultures associated with Pima plants of any chlorid content are seen to be to some extent variable. It will also be noted that there is a tendency for the chlorid content of Upland plants to shift upwards in passing from the lower to the higher classes of chlorid content in the Egyptian (Pima) cotton.

TABLE IV.—Comparison between the chlorid contents of Pima Egyptian and Meade Upland cotton as grown at Sacaton, Ariz., 1922

Totals	1	1	1	1	—	3	7	3	9	4	7	1	2	1	2	3	—	—	1	47
Chlorid in Meade																				
11.20																				
10.85																				
10.50																				
10.15																				
9.80																				
9.45																				
9.10																				
8.75																				
8.40																				
8.05																				
7.70																				
7.35																				
7.00																				
6.65																				
6.30																				
5.95																				
5.60																				
5.25																				
4.90																				
4.55																				
4.20																				
3.85																				
3.50																				
3.15																				
2.80																				
	4.90	5.25	5.60	5.95	6.30	6.65	7.00	7.35	7.70	8.05	8.40	8.75	9.10	9.45	9.80	10.15	10.50	10.85	11.20	47
Chlorid in Pima																				

<sup>12</sup>Possibly the greater variability of chlorid content in this experiment is due to the facts (a) that the chlorid content is on the average higher in this culture, and that (b) the analyses are based on samples of tissue collected from individual plants instead of a considerable number of plants.

Leaving this point for the moment and considering merely the relative amounts of chlorids in the two types of cotton, we may note that if the chlorid content of the two varieties were sensibly the same, the frequencies corresponding to given values of chlorid content in the two forms under consideration should be distributed at random about the "diagonal cell"—i. e., the line of cells corresponding to identical values on the scales of chlorid content for the Egyptian and the Upland varieties under comparison, of these tables. Thus the heavy bars mark off the chlorid content which the Upland cottons would have had if they had been as rich in chlorids as the Egyptian form.

The fact that without exception in the illustrative table, and almost without exception in the unpublished tables for the other series the chlorid content of the Upland forms lies below the diagonal cell which indicates equal chlorid content in the two forms, shows how conspicuously and definitely the Egyptian and Upland types are differentiated with respect to their content of this anion.

From such a table of double entry the differences in the chlorid contents of the associated Egyptian and Upland plants may be readily determined. This has been done for the comparison between Pima and Meade and Pima and Acala cultures in 1922 and for Pima and Lone Star cultures grown in 1923. The results are set forth in Table V. These entries show that practically without exception the individual comparisons show a higher chlorid content in the Egyptian than in the Upland cotton plants.

TABLE V.—*Differences in chlorid content (grams per liter in 0.25 gm. class) of leaf tissue fluids of associated Egyptian and Upland cotton plants*

Difference in chlorid content (Egyptian less Upland)	Pima Egyptian and Meade Upland		Pima Egyptian and Acala Upland		Pima Egyptian and Lone Star Upland		Difference in chlorid content (Egyptian less Upland)	Pima Egyptian and Meade Upland		Pima Egyptian and Acala Upland		Pima Egyptian and Lone Star Upland	
	First series	Sec-ond series	First series	Sec-ond series	First series	Sec-ond series		First series	Sec-ond series	First series	Sec-ond series	First series	Sec-ond series
-0.50				1			+2.25	4	12	1	15	3	9
-0.25							+2.50	1	9	1	4	3	11
0.00	1			1			+2.75		7		2		6
+0.25	1		8				+3.00	1	5				3
+0.50	4		9	2	3		+3.25		4		2		4
+0.75	4		14		6	1	+3.50		7				1
+1.00	11	1	9	3	15	2	+3.75						1
+1.25	10	2	12	6	7	3	+4.00		1				
+1.50	13	2	4	15	19	9							
+1.75	8	7	5	8	8	9							
+2.00	11	10	6	9	4	9		67	67	69	68	68	68

As noted above, there is an evident tendency for the variable chlorid content of the Upland plants to be larger when associated with Egyptian plants of a higher class of chlorid content. This is more strikingly evident in some of the unpublished tables than in the illustrative one given (Table IV). This may be most readily explained as due to both Egyptian and Upland plants being influenced in the same manner by external conditions, presumably in this case by variation of chlorid concentration in the soil. Thus we have a further illustration of substratum heterogeneity as a factor influencing the behavior of plants in field plots (3, 4).



TABLE VI.—Correlation between the chlorid content of associated plants or groups of plants of Egyptian and Upland cotton as grown at Sacaton, Ariz., in 1922-23

	Correlation for first series		Correlation for second series.	
	Correlation coefficient and probable error $r \pm E_r$	Ratio of correlation to probable error $r/E_r$	Correlation coefficient and probable error $r \pm E_r$	Ratio of correlation to probable error $r/E_r$
First comparison of Pima Egyptian and Meade and Acala Upland cotton, 1922 (B):				
Correlation between Pima and Meade.....	0.6702 $\pm$ 0.0454	14.76	0.6029 $\pm$ 0.0524	11.50
Correlation between Pima and Acala.....	.6844 $\pm$ .0432	15.85	.6030 $\pm$ .0520	11.59
Second comparison of Pima Egyptian and Meade Upland cotton, 1922 (C): Correlation between Pima and Meade.....	.5144 $\pm$ .0723	7.11	-----	-----
Comparison of Pima Egyptian and Lone Star Upland cotton, 1923 (D): Correlation between Pima and Lone Star.....	.3372 $\pm$ .0725	4.65	.1698 $\pm$ .0794	2.14

The coefficients of correlation,  $r$ , measuring the relationship between the chlorid content of associated plants or subrows of plants, are given with their probable errors in Table VI.

The coefficients for the cultures of 1922 are of about the same order of magnitude as those already published for osmotic concentration and specific electrical conductivity in cultures of Pima Egyptian, Meade and Acala Upland and hybrid cotton grown at Sacaton in 1921 (5). The constants for Pima Egyptian and Lone Star Upland cotton studied in 1923 are much lower. This is presumably due to the greater uniformity of the half of the plot upon which this culture was grown.

The fact that the chlorid content of associated plants is correlated is not a matter of merely physiological interest. It must be considered in determining the statistical significance of the differences between the varieties. The fact that the variability of the constants is due largely to the heterogeneity of the substratum makes it essential that the constants for any two varieties compared be based on plants which have grown in the closest association practicable, and that the constants compared comprise only individuals of the same pair.

Since the two types to be compared were grown in close association in a large number of subplots distributed over the cultural area, both were subjected to the influence of similar soil conditions. This tends to reduce the probable errors of the difference between the mean chlorid content of the Egyptian and Upland types as based on the whole series of determinations available for each culture and series of samples. These probable errors may be calculated from the standard deviation of the difference between associated determinations as obtained from the formula

$$\sigma^2_{(p-u)} = \sigma^2_p + \sigma^2_u - 2r_{pu}\sigma_p\sigma_u,$$

or by actually determining the individual differences between the Pima and Upland determinations of the associated plants and calculating the standard deviations of these differences directly.

Table VII presents the mean chlorid content in terms of grams of Cl per liter of tissue fluids for the Egyptian and Upland varieties compared in the various cultures.  $N$  denotes the number of pairs of determinations. The probable errors of the means of the individual series have been calculated by the usual formula. The standard deviations are not published since variability of chlorid

content is not discussed in this place. The probable errors of the differences have been calculated with due regard to the correlation between associated plants by the formula given above.

In all comparisons the average chlorid content of the Egyptian cotton is higher than that of the associated Upland plants. The differences are many times as large as their probable errors and are unquestionably significant.

TABLE VII.—Mean chlorid content (in terms of grams of Cl per liter) of tissue fluids of Egyptian and Upland cotton as grown at Sacaton, Ariz., in 1922-23

	N	Mean chlorid content for Egyptian cotton	Mean chlorid content for Upland cotton	Difference between Egyptian and Upland cotton		
				Absolute difference and probable error	Ratio of absolute difference to probable error	Percentage difference
First comparison of Pima Egyptian and Meade and Acala Upland cotton, 1922 (B):						
First series, July 25 to Aug. 9—						
Comparison of Pima and Meade.....	67	4.7388±0.0642	3.1082±0.0538	1.6306±0.0488	33.4	52.5
Comparison of Pima and Acala.....	69	4.7355±.0626	3.6848±.0440	1.0507±.0457	23.0	28.5
Second series, Aug. 28 to Sept. 4—						
Comparison of Pima and Meade.....	67	5.2276±.0676	2.7799±.0514	2.4478±.0550	44.5	88.1
Comparison of Pima and Acala.....	68	5.2352±.0647	3.4522±.0501	1.7831±.0528	33.8	51.7
Second comparison of Pima Egyptian and Meade Upland, 1922 (C): First series, Aug. 21 to Aug. 27—Comparison of Pima and Meade.....	47	7.8862±.1266	4.2670±.1120	3.6191±.1183	30.6	84.8
Comparison of Pima Egyptian and Lone Star Upland cotton, 1923 (C):						
First series, July 29 to Aug. 14—Comparison of Pima and Lone Star.....	68	2.4412±.0403	1.0515±.0239	1.3897±.0396	35.1	132.2
Second series, Aug. 18 to Aug. 31—Comparison of Pima and Lone Star.....	68	3.3088±.0477	1.1434±.0302	2.1654±.0520	41.7	189.4

When the differences are expressed in percentages of the chlorid content of the Upland type instead of in terms of grams of chlorid (as Cl) per liter of tissue fluid, the true significance of the differentiation with respect to chlorid content becomes fully apparent. The Egyptian cotton is from 28.5 to 189.4 per cent richer in chlorids than the Upland cotton grown as a control.

The causes of these variations in the differences from culture to culture must be a subject for further investigation. In passing we may note that in the comparison involving Pima Egyptian and both Meade and Acala Upland cottons the difference between Pima and Meade is greater than that between Pima and Acala in both the first and second series of determinations. We may also point to the fact that in both cultures in which two series of determinations were made the differentiation of the Egyptian and the Upland types is greater in the series of analyses made later in the season. Both of these problems will receive more detailed consideration later.

## SUMMARY

This paper presents the results of a detailed comparison of the chlorid content of the leaf tissue fluids of Egyptian and Upland cotton.

Several hundred determinations based on an American Egyptian variety, Pima, grown in comparison with one or more of the Upland varieties, Acala, Meade, and Lone Star, show that almost without exception the chlorid content is higher in the tissue fluids of the Egyptian than in those of Upland cottons.

The means for seven series of determinations show the chlorid content of the Egyptian type to be from 28 to 189 per cent higher than that of the Upland type.

This is clearly one of the factors in determining the higher osmotic concentration and specific electrical conductivity demonstrated in an earlier paper (5) on the tissue fluids of two of these varieties.

The higher chlorid content may indicate a greater capacity of the Egyptian type for growth on saline land.

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# THE RESISTANCE OF OAT VARIETIES TO STEM RUST <sup>1</sup>

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## INTRODUCTION

An essential preliminary to breeding for rust resistance is a knowledge of the resistant varieties already in existence. An additional requirement, in the case of cereal rusts at least, is a knowledge of the physiologic specialization within the species of rust under consideration.

Since the discovery by Stakman and Piemeisel (20) <sup>2</sup>, in 1917, of a second race of wheat stem rust, differing in its range of power of infection from the rust which they had been using, our knowledge of physiological races in cereal rusts has developed rapidly. In 1918 Levine and Stakman (8) reported a third race, and Melchers and Parker (11) still another. In 1919 Stakman, Levine, and Leach (19) distinguished about 12 physiological races and, in 1922, Stakman and Levine (16) reported a total of 37. Miss Newton (13), in 1922, made a survey of wheat stem rust in Canada and found there 14 of the 37 physiological races which are known to occur in the United States.

The discovery of physiologically distinct races of wheat stem rust led to similar studies of other cereal rusts. Hoerner (7), in 1919, working on *Puccinia coronata* Cda., tested many specimens of rust on certain varieties of oats chosen as differential hosts, and concluded that probably there are four physiological races of crown rust of oats. Melhus, Dietz, and Willey (12), in 1922, tested a wide range of grass hosts with crown rust from cultivated oats and wild grasses and distinguished four physiological races. They state (12, p. 234) that "*Avena sativa* was a common host with varying degrees of infection for all the forms of crown rust studied."

In 1923 Mains and Jackson (10), in a preliminary report of their work on leaf-rust of wheat, *Puccinia triticina* Erikss., state that this rust consists of at least 12 races. Levine and Stakman (9), 1923, find "at least two and probably three distinct biologic forms of *Puccinia graminis secalis*."

Stakman, Levine, and Bailey (17, 18), in a preliminary report and a later report on oat stem rust, *Puccinia graminis avenae* Erikss. and Henn., state that "four, and perhaps five, biologic forms of this rust can be identified by their action on Victory (C. I. 1145), White Russian (C. I. 1614), and Monarch Selection."

Parker (15) has published a comprehensive review of the literature on the rusts of oats.

A few extensive tests have been made in the search for varieties of oats that are resistant to stem rust. Vavilov (21, 22), working in Russia, inoculated 450 varieties of wild and cultivated oats with stem rust and found but two that were not fully susceptible. *Avena sativa* var. *brunnea* Kcke. was graded 2½ (using a scale in which 0 represents immunity and 4 complete susceptibility) and *Avena*

<sup>1</sup> Received for publication March 26, 1924. Cooperative investigations between the Agricultural Experiment Station of the University of California and the Bureau of Plant Industry, U. S. Department of Agriculture.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," pp. 718-719.

*diffusa* var. *montana* Al. was graded 3. The rest were uniformly 4. These two are low, slender-strawed varieties of small value. Vavilov pointed out that black rust of oats is a weakly specialized fungus which lives freely on several genera besides *Avena*, and states that "we come to a simple statistical conclusion as to the very slight probability of plant breeders finding oats resistant to black rust."

Parker (14) tested 120 strains of oats with both crown and stem rusts. Only White Tartarian and Ruakura Rustproof showed pronounced resistance to stem rust. Later, Durrell and Parker (1) published the results of a five-year test of about 200 lots of oats. The following were found resistant to stem rust: White Russian (White Tartar), Ruakura, Green Russian, *A. sativa grisea*, *A. orientalis mutica*, and *A. barbata*.

A beginning has been made in the breeding of oats for resistance to stem rust. Garber (4, 5) made crosses between White Russian (White Tartar), a rust-resistant oat with side panicle, and Minota and Victory, susceptible forms with open panicle. He found that "rust resistance is inherited as a dominant character depending on a single factor difference for its expression," and that rust reaction and panicle type are independent in their inheritance. Griffiee (6) reports the rust test of the  $F_2$  generation of these crosses. Of the resistant  $F_2$  families, one-third bred true for resistance in the  $F_2$  and two-thirds segregated.

TABLE I.—Result of inoculation of oat varieties with stem rust at Berkeley, Calif., in the summer of 1920, as measured on a scale of 0 to 4, in which 0 represents immunity and 4 represents complete susceptibility<sup>a</sup>

Host	Lot No.	C. I. No.	Greenhouse tests		Field tests	
			First	Second	First	Second
<i>Avena brevis</i> Roth.						
var. Do.....	R 134d		4	3+	2+	3-
Do.....	R 142a		4-	3+	3-	4-
Do.....	R 289a	1783	3	3	3+	3+
<i>Avena fatua</i> L.						
var. Do.....	R 290a	1779	4	4	3+	4-
var. California Wild Oat. (Lemmas slightly 2-toothed).....	C 1024		4-	3+	3+	4
<i>Avena nuda</i> L.						
var. <i>chinensis</i> .....	R 30i		4-	4-	3	4-
var. Chinese Hull-less.....	W 686	298	4-		3	
var. <i>inermis</i> .....	R 294a	1768	3+	4	3	3+
var. Liberty.....	R 292a	845	4-	3+	3	4-
Do.....	R 293a	1769	3	4-		3+
Do. (Differs in having geniculate awns).....	C 1000			3		4
<i>Avena sativa</i> L.						
var. Abundance.....	R 269a	731	4-		4-	3
var. Abyssinian.....	R 303a	1747	4	4	4-	3
var. Albion.....	R 270a	729	4-	3	3	3
Do.....	R 304a	729	4-	4	3	4-
Do. (Iowa 103).....	I 51	729		4-		3-
var. <i>aristata</i> .....	R 35g		3	3+	3-	4-
var. <i>aurea</i> .....	R 7s		4-	3+		4-
Do.....	R 328a	1772	4-	4	3	4-
var. Awnless Probsteler.....	R 114d	1888	4-	3	3+	4
var. Banner.....	R 271a	160	4-	3+	4	4
Do.....	C 1031		3+	3-	3+	4-
Do.....	W 764	1191	4	3+	4	4-
var. Belyak.....	R 115e	1899	4-	3	3+	3+
Do.....	R 306a	1725	4	4	4-	4

<sup>a</sup> In the tables, the lot numbers indicating the different selections are preceded by various capital letters, which indicate the sources from which the seed was received. The lot numbers following the letter "C" are accession numbers of the California Agricultural Experiment Station. The lots designated by the letter "I" were received from the Iowa Agricultural Experiment Station and the numbers are nursery row numbers or selection numbers of the Department of Botany and Plant Pathology. The one lot bearing the letter "K" was received from the Kansas Agricultural Experiment Station. The numerous lots bearing the letter "R" were received from Dr. G. M. Reed, then pathologist in charge of smut investigations, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, and the numbers are those used by him in the oat-smut nursery. The lots following the letter "W" were received from the Washington Agricultural Experiment Station, and the numbers are accession numbers of that station.

TABLE I.—Result of inoculation of oat varieties with stem rust at Berkeley, Calif., in the summer of 1920, as measured on a scale of 0 to 4, in which 0 represents immunity and 4 represents complete susceptibility—Continued

Host	Lot No.	C. I. No.	Greenhouse tests		Field tests	
			First	Second	First	Second
<i>Avena sativa</i> L.—Continued						
var. Big Four	R 272a	179	4	4	4—	4
Do.	R 307a	1641	4—	4	3+	4—
Do.	W 1036		3	3+	4	4—
var. Black. (Received as <i>Avena sterilis</i> )	R 295a	691	3—	3+	3	4—
var. Black American	R 185a	549	4	3	3+	4—
var. Black Diamond	R 116e	1878	3	3	3+	3
var. Black Mesdag	R 117e	1877	4—	4—	3	3
var. <i>brunnea</i>	R 81		4—	3+	3	4—
var. Canadian	R 119e	1892	3+	4—	4	3+
Do. (Barley Oat)	W 1179	1806	4—	3+	4	3—
var. Caucasus	R 279a	1754	3+	4—	4	4—
var. C. I. 602 (of Etheridge)	R 145a	602	4—	3—	3	3+
var. C. I. 603 (of Etheridge)	R 146a	603	4	3—	3—	4
var. Culberson. (The three lots differ morphologically)	R 192a	273	4	4—	4	3
Do.	R 193	273-V		4—	4	4—
Do. (Listed as Culberson Winter)	C 1022		3+	4	4—	3—
var. Danish	C 1032		3—	2+	4	4—
var. Danish Island. (Awns vary in the 4 lots)	R 149a		4	3+	4—	4
Do.	R 309a	1669	4—	4	4	4—
Do.	R 310a	1666	4—	4	4—	4—
Do.	R 311a	1684	4—	4	3	3
var. Dryland	W 767	1193	3—	3—	4	3+
var. Dun	C 1025		3—	4	4—	3+
var. Dwarf Culberson	R 196a	748	4—	3	4—	3
var. Early Champion	R 197a	1623	4	4—	3+	3+
var. Early Gothland	R 162a		4	3+	3+	4—
var. Early Mountain No. 2. (Probably Golden Drop)	R 273a	656	4—	4—	3+	4—
var. Garton 473. (Received as Garton's Regenerated Yelder)	C 1033		3+	2+	4	3+
Do. (Received as <i>Avena sativa orientalis</i> )	R 297a	1613	3+	4—	4—	3
var. Golden	R 313a	1750	3+	4	4—	3+
var. Golden Beauty	R 314a	1748	4	4	4—	3+
var. Golden Fleece	R 315a	1749	3+	4	4	4—
var. Golden Rain. (Probably Golden Drop)	R 275a	493	4—	4—	3+	3
var. Golden Rustproof	R 316a	1751	4	4	4—	3
Do.	W 1055	1929	3+	4—	4	3+
var. Gray. (Probably a spreading-panicked segregate from Garton Gray)	W 663		4—	3	4	4
var. Green Russian	R 121e		3+	3	3	4
Do. (Contains a resistant strain)	R 203a	1683	3+ & 1—	3+ & 1—	3—	1— & 4
Do. (Contains a resistant strain)	R 276a	827	4	4— & 1	3+	1— & 3+
Do.	I 91-4			1—		2—
var. Guyra. (Received as Gluysa)	C 1019	2034	4—	4	3—	3
var. Hatchett. (Received as Black Oat)	R 296a	838	3	3+	4	3+
var. Hay	R 317a	1622	3+	4—	2+	3
var. Heavyweight Champion	R 318a	1690	3	4	3+	4
var. Highland	W 772		2	2+	3	4—
var. Joannette. (May be X type of infection)	R 125e	1880	3 & 0	3 & 1—	1 to 2+	1 to 2+
var. June	R 157a	1902	4	3	3—	4
var. Kherson	C 1021		3+	4	2	3—
Do.	W 1047	1198	3+	3	2+	4—
Do. (Wisconsin Pedigree No. 156)	C 1029		4—	4—	4—	3—
var. Kherson Selection (of Etheridge)	R 159a	1905	4	3	2—	3+
var. <i>Krausei</i>	R 329a	1771	3+	4	4	4—
var. Lincoln	C 1015		3+		3+	4—
var. Long White	C 1018		3—	4	4—	4—
var. Maine No. 340	W 1197	1935	3+	4	3+	3
var. Minnesota No. 26	R 278a	721	3+	4—	3+	4
var. Monarch Selection (of Etheridge). (Received as Monarch)	R 161a	1879	4	3+	4	4—
var. Montana	R 285a	1761	3	3+	4—	4
var. Mortgage Lifter	W 775		3	3	3+	3+
var. <i>mutica</i> . (Awns vary in the different lots)	R 42k		4—	3	3+	4
Do.	R 43c		3+	3+	4—	4—
Do.	R 49e		3—	2+	4—	4
Do.	R 51g		3	3	3—	3+

TABLE I.—Result of inoculation of oat varieties with stem rust at Berkeley, Calif., in the summer of 1920, as measured on a scale of 0 to 4, in which 0 represents immunity and 4 represents complete susceptibility—Continued

Host	Lot No.	C. I. No.	Greenhouse tests		Field tests		
			First	Second	First	Second	
<i>Avena sativa</i> L.—Continued							
var. <i>mutica</i> . (Awns vary in the different lots).....	R 52g		3—	3	4—	3+	
Do.....	R 53e		3	3	4		
Do.....	R 330a		4	4	4	4	
Do.....	R 331a	1716	4—	4	4—	4—	
var. Nebraska No. 21.....	R 280a	841	3+	4—	3—	3+	
var. New Market.....	W 1048		3—	2+	4—	4	
var. <i>nigra</i> . (Closely resembles Black Mesdag).....	R 70g		4	3	4	4—	
var. North Finnish.....	R 123e	1882	4—	3+	4	3+	
Do.....	C 1002	1801	3+	3+	3+	4	
var. Palouse Wonder.....	W 748		3+	4—	4	4—	
var. Potato. (Early Mountain).....	W 761	1192	3+	3+	3—	4—	
var. <i>praeagravis</i> .....	R 62f	1773	4—	3		4—	
Do.....	R 332a	1773	4—	4—		3	
Do.....	R 333a	1775	3	3+	4	4—	
var. President. (Closely resembles Swedish Select).....	R 319a	1629	3—	4	4	4—	
var. Richland.....	R 281a	787	0+	1—	1	1—	
Do.....	R 320a	787	0+	1—	1—	1—	
var. Roosevelt. (Closely resembles Swedish Select).....	R 321a	1632	3	4—	4—	3	
var. Rossman.....	R 322a	1688	3+	4	4—	3+	
var. Ruakura.....	I 73	791		1—		1+	
var. Sandwich. (Garton No. 5).....	W 758	1194	3—	4—	3+	3	
var. Scottish Chief.....	R 124e	1901	4—	3	2	4	
var. Siberian.....	R 282a	741	4—	4—	3	4—	
Do.....	R 323a	1712	3	4	4	3	
var. Silvermine.....	R 220a	659	4	3+	4	4	
Do.....	I 20			3		4	
var. Sixty-Day.....	R 223a	165	4	3	2	2	
Do.....	C 1004		3+	4—	3+	2+	
Do.....	W 661	165	3+	3	3—	2+	
var. Sixty-Day Selection (of Etheridge).....	C 1010	1906	3+	3—	3+	3—	
var. Snoma. (Received as Snoma Winter. Seed mixed, and identification of this resistant strain doubtful).....	C 274		1	1	1—	1	
var. Swedish Crown.....	C 1006		3	3—	4—	4	
Do.....	W 1035		3	3+	4—	4—	
var. Swedish Select.....	R 226a	134	4—	3—	4	4	
Do.....	R 228a	552	4	4—	3	3	
Do.....	R 231a	1634	3+	4—	3—	3+	
Do.....	W 662		4—	3	3	3	
Do. (Ligowa).....	R 227a	1740	4—	3+	4—	4—	
Do.....	W 1088	1740	3	3	3+	3	
Do.....	I 59			4—		4—	
var. Swedish Victory.....	C 1007		4—	4	4—	4	
var. Tobolsk.....	R 122e	1893	3+	3	4	4	
Do.....	R 232a	1709	3—	4—	4—	4	
var. <i>trisperma</i> .....	R 334a	1776	3—	4	3	3+	
var. Victor.....	R 126f	1875	4—	3	4	4—	
Do. (Received as Black Tartarian).....	C 1028		3—	4—	4	4—	
Do. (Received as Black Alaska).....	W 1023		3	3+	4	3+	
var. Victory.....	W 780		3+	4—	3+	3+	
Do.....	R 283	560	4—	4—	4—	4	
var. Vogt New Era.....	C 1023		3	4—	3+	3+	
var. Wernicke's Golden.....	R 324a	1672	4—	4—	4	3	
var. White Bonanza.....	R 325a	1650	4	4	3+	3	
var. White Maine.....	R 326a	1651	4—	4	4—	4—	
var. White Queen.....	R 327a	1700	4	4	3	4	
var. Winter Turf. (Lots of Winter Turf differ in kernel color and awns).....	R 169a		4—	3+	3—	3	
Do.....	R 235a	195-15	3—	2+	3+	4	
Do.....	R 236a	274-20	3	2+	3—	4—	
Do.....	R 237a	673	4—	4—	3	4	
var. Winter Turf. (Received as Gray Winter).....	W 1030		3—	3—	4	4	
var. Black X Sixty-Day.....	W 1186		4—	3+	3	3+	
<i>Avena sativa orientalis</i> L.							
var. Black Tartarian. (Received as Black Diamond).....	R 308a	1763	4—	4	3+	4—	
Do.....	W 762		3	3+	3+	3—	
var. Eclipse. (Mixed, containing resistant strain of White Tartar type).....	R 274a	843	3	3+ & 1—	4—	1— & 4	

TABLE I.—Result of inoculation of oat varieties with stem rust at Berkeley, Calif., in the summer of 1920, as measured on a scale of 0 to 4, in which 0 represents immunity and 4 represents complete susceptibility—Continued

Host	Lot No.	C. I. No.	Greenhouse tests		Field tests		
			First	Second	First	Second	
<i>Avena sativa orientalis</i> L.—Continued							
var. Eclipse	R 312a	843	4—	4	4	3+	
var. Garton 748	R 298a	1612	4—	4—	4—	3	
Do	R 109e	1863	4	4—	4	3	
var. Garton Gray	R 172a	—	4—	3+	4	4—	
var. German type	R 335a	—	4	4	4—	4—	
Do	R 336a	—	4—	4	4	4	
Do	R 337a	—	4	4	4	4	
var. Golden Giant	R 173a	1866	4	4—	3+	3+	
var. Green Mountain (White Tartar type)	R 110e	1872	1	1	1	1—	
var. Improved Roberts	C 1017	—	3+	4—	4—	4—	
var. Long's White Tartar (White Tartar type)	C 1026	—	1+	1+	1+	1—	
var. <i>mutica</i> (White Tartar type)	R 32i	—	1	1	2—	1	
var. <i>obtusata</i>	R 33i	—	4—	4—	3—	3	
var. <i>setosa</i>	R 302a	1618	4	4—	2	4—	
var. Short Tartarian (Sparrowbill type)	C 1027	—	3+	4—	4	3—	
var. Sparrowbill	R 111e	1867	4—	3	3+	4	
Do	C 1030	1812	4—	4—	3+	3	
var. Storm King	R 112d	—	3+	4—	4	4	
Do	C 1001	—	3+	4	3+	4—	
var. White Egyptian (Sparrowbill type)	R 300a	1605	2	4	4—	4	
var. White Tartar. (Mostly received as White Russian)	R 284a	551	1—	1	0+	1+	
Do. (Received as Improved Snoma Winter)	C 1016	—	1+	1	1	1	
Do	R 301a	1614	1—	1+	1	1	
Do	I 98-4	—	—	1—	—	1—	
Do	I 1024	—	—	1	—	1—	
Do	I 1024-5	—	1+	1	2—	1—	
Do	I 1024-7	—	1	1	1	0+	
Do. (Received unnamed)	K 6096	—	1	—	1—	—	
Do	R 174a	—	1—	1	1—	1+	
Do. (Received as Tartarian)	C 1020	—	1+	1+	—	1	
Do. (Received unnamed; has more awns than normal)	R 299a	1615	3+	4—	4—	4—	
Do	W 739	441	1	1	—	0+	
<i>Avena sterilis</i> L.							
var. Bathurst No. 4	C 1014	1810	3	4—	3+	4—	
var. Burt	R 67n	—	4	3+	2+	2+	
Do	R 74k	—	4	4—	3	3	
Do	R 175a	—	4—	4	2+	2+	
Do	R 253a	—	4—	4	3	3+	
Do	C 1005	1798	4—	4—	3—	3+	
Do	I 25	—	3+	3+	3—	3—	
var. Burt Selection	C 1011	—	3+	4	4—	3+	
var. California Red	C 1003	1026	4—	4	3	4—	
var. Early Ripe	R 75j	—	4—	3+	4	4	
Do	I 26	—	—	3—	—	3—	
var. Fulghum	R 257a	708	4—	4—	3	—	
Do. (Received as King)	R 129f	850	4—	3+	2+	2+	
var. Improved California Red	C 1013	1811	4—	4	3—	3+	
var. Italian Rustproof	R 258a	388-6	3	3+	3+	4—	
Do	R 259a	388-6	—	3+	—	3	
var. <i>ludoviciana</i> Dur.	R 291a	1781	3+	4	3+	4	
var. <i>nigra</i> . (Received as Black Oat)	C 1008	—	4	4	4—	4	
Do	R 130c	—	3	3+	3—	3	
var. No. 356	C 1009	—	4	4	3—	3—	
var. Red Algerian	C 286	286	3+	4	3+	3+	
var. Red Rustproof	R 263a	—	4—	4—	3	3	
Do	R 265a	—	4—	3+ to 4—	3	3+	
Do	I 67	—	—	3	—	3—	
var. Sterilis Selection (of Etheridge)	R 132e	1859	4—	4—	3—	3	
var. Sunrise	C 1012	1799	3—	—	3+	3+	
var. Red Rustproof	W 768	—	3	3+	3	2+	
var. Turkish Rustproof	R 267a	356	4—	4—	3+	3+	
Do	R 268a	356-12	4—	—	3+	4	
var. (Received unnamed)	R 28k	—	4—	4	3+	4	
<i>Avena strigosa</i> Schreb.							
var	R 29j	—	4—	4—	3+	2	
var	R 133e	1782	4—	4	3—	3—	
var. (Received as <i>Avena barbata</i> )	I 119-3	—	—	2+	—	3	
Do	I 119-4	—	3	3+	3—	3+	



## INVESTIGATIONS

Two distinct experiments were conducted. In the first a single rust, collected at Berkeley, Calif., was used. In the second, nine different collections of rust, from as many localities in the State, were used for inoculations.

## EXPERIMENTS WITH ONE RUST IN GREENHOUSE AND FIELD

The varieties of oats tested were obtained from the following sources:

	Varieties
United States Department of Agriculture.....	140
California Agricultural Experiment Station.....	36
Washington Agricultural Experiment Station.....	26
Iowa Agricultural Experiment Station.....	14
Kansas Agricultural Experiment Station.....	1
Total.....	217

The lists included duplicates of many of the varieties. Some of the plants bearing the same name were very similar morphologically, but others differed somewhat. All were included in the tests. In other cases, plants bearing different names were alike. Etheridge's "Classification of the Varieties of Cultivated Oats" (3) was used in studying the material and any changes that were made in the names were in conformity to his descriptions. Each change in the name of a variety is recorded.

The oat stem rust used in 1920 to obtain the results shown in Table I was found growing on cultivated oats in the botanical garden at Berkeley in the winter of 1919-20. It was cultured on red oats in the greenhouse. Square paper pots were placed in flats holding four rows of pots, with 10 in each row. Each variety occupied one row in a flat, a single seed being sown in each of the 10 pots. As the available greenhouse space was not great enough to accommodate all of them at once, they were grown in successive lots. The seedlings were inoculated when they were about 10 days old and were kept in a damp chamber 48 hours. After two weeks the rust was studied and recorded, and the rusted leaves were packeted, labeled, and pressed. The seedlings were then transplanted to plats in the botanical garden. The first lots were set out on the seventeenth of February and the last on the twenty-seventh of March. The series was then repeated, the second set being about six weeks later than the first.

When half grown these plants were subjected to a heavy artificial epidemic of the same rust. For this purpose urediniospores were grown in the greenhouse. These spores were put into tap water and applied to the plants either by the use of an atomizer or by rubbing the culms with moistened fingers. As the epidemic developed on the older plants spores were transferred by the same method from the older to the younger plants. When nearing maturity, rusted specimens of each variety (including at least one complete culm) were studied, recorded, packeted, and pressed. Thus, each variety was subjected to four rust tests, two in the greenhouse and two in the field. The rust was measured on a scale of 0 to 4, in which 0 is immunity and 4 is complete susceptibility.

Eriksson and Henning (2), in "Die Getreideroste" (1896), used in their tables a scale of 1 to 4 for grading rust. Rust-free material was recorded in a column headed "rein," 1 indicated traces of rust, 2 and 3 larger amounts, and 4 abundance of rust. Their estimates were based largely on the quantity of rust found.

As used in this paper, the scale of 0 to 4 is not based on quantity of rust but on the size and appearance of individual uredinia. The types of infection as defined

by Stakman and Levine (16, p. 5) and their associates have been used in these studies. They are as follows:

0. IMMUNE.—No uredinia developed; hypersensitive flecks usually present, but sometimes there is apparent absolutely no trace of mycelial invasion in the host tissues.

1. VERY RESISTANT.—Uredinia minute and isolated; surrounded by sharp, continuous, hypersensitive, necrotic areas.

2. MODERATELY RESISTANT.—Uredinia isolated and small to medium in size; hypersensitive areas present in the form of necrotic halos or circles; pustules often in green, but slightly chlorotic, islands.

3. MODERATELY SUSCEPTIBLE.—Uredinia medium in size; coalescence infrequent; development of rust somewhat subnormal; true hypersensitiveness absent; chlorotic areas, however, may be present.

4. VERY SUSCEPTIBLE.—Uredinia large, numerous, and confluent; true hypersensitiveness entirely absent, but chlorosis may be present when cultural conditions are unfavorable.

X. HETEROGENEOUS.—Uredinia very variable, apparently including all types and degrees of infection on the same blade; no mechanical separation possible; on reinoculation small uredinia may produce large ones, and vice versa. Infection ill defined.

Table I shows that the great majority of the varieties tested are susceptible to the oat stem rust used (usually grading from 3— to 4), a few are highly resistant (grading from 1— to 1+), and not one is fully immune (grade 0).

The oats fall naturally into two well-defined groups on the basis of rust resistance, and there are few intermediates. Some of the slender varieties with weak straw may not show as large uredinia or as abundant spore production as the stouter-stemmed varieties with greater substance, but they may be quite as susceptible. It is quite possible, too, that of two varieties showing equal susceptibility in the seedling tests in the greenhouse one may mature earlier than the other and so escape the worst ravages of the disease. This is quite distinct from the question of susceptibility.

Examples of the susceptible group are given in Plate 1. A is a specimen of *Avena sativa* L. (Silvermine, I 20) and B is *Avena sativa orientalis* L. (Short Tartarian, C 1027), a type resembling Sparrowbill. In both varieties the fungi make an abundant growth and produce well-developed uredinia often confluent into broad streaks several inches long and bearing urediniospores in abundance. In sharp contrast to this are the resistant forms. The specimens photographed (Pl. 2) belong to *Avena sativa orientalis* and are of the White Tartar (White Russian) type. A was listed as Long's White Tartar (C 1026) and B as White Tartar (C 1020). The fungus enters these hosts as readily as the susceptible varieties. In fact, each specimen is peppered with hundreds of minute uredinia, giving it a speckled appearance, but the development of each fungus is sharply limited, the sorus remains minute, and its spore output is negligible. Examination shows that it frequently contains teliospores.

Resistant varieties were found only in *Avena sativa* L. and its subspecies *A. sativa orientalis* L. All the available lots of *A. brevis* Roth., *A. fatua* L., *A. nuda* L., *A. sterilis* L., and *A. strigosa* Schreb. were found to be susceptible.

For convenience the data on the resistant varieties of *Avena sativa*, from Table I, are presented separately in Table II. The two lots of the variety Richland are similar. The plants are erect, with fine, short culms, narrow leaves, and small sparsely-branched panicles of the general type of Kherson. The glumes are short, the kernels light yellow and awnless, and there is an occasional three-kerneled spikelet. The reaction to rust is 0+ to 1, so far as observed.

Four lots of Green Russian are included in the varieties tested but only one lot proved uniformly resistant to stem rust. The first of these lots (see Table I) contained only susceptible plants; the second and third contained a small percentage of markedly resistant ones in addition to those susceptible; and the fourth, Iowa 91-4, appears to be a selection containing only the resistant strain.

In the subspecies *Avena sativa orientalis* L., 15 lots proved resistant. They were received from several sources under seven different names. Close examina-

tion of the plants shows that they differ only in minute morphological details. They are all of the White Tartar (White Russian) type. The data on these, from Table I, are given separately in Table III.

The seed of the variety called Eclipse was mixed and produced, in addition to the resistant plants, others which were markedly susceptible.

TABLE II.—Data on varieties of *Avena sativa* resistant to oat stem rust

Host	Lot No.	C. I. No.	Greenhouse tests		Field tests	
			First	Second	First	Second
<i>Avena sativa</i> L.:						
var. Green Russian.....	I 91-4			1-		2-
var. Richland.....	R 281a	787	0+	1-	1	1-
Do.....	R 320a	787	0+	1-	1-	1-
var. Ruakura <sup>a</sup> .....	I 73	791		1-		1+
var. Snoma <sup>b</sup> .....	C 274		1	1	1-	1

<sup>a</sup> The variety Ruakura has the same general habit as Richland but has much longer glumes. The kernel is whitish and the awns are few and delicate or wanting. The rust test runs from 1- to 1+.

<sup>b</sup> Snoma is a gray oat of the Winter Turf type, more or less spreading, with a narrow leaf, narrow stiff panicle, and a few geniculate awns.

TABLE III.—Data on varieties of *Avena sativa orientalis* of the White Tartar (White Russian) type which are resistant to stem rust

Host	Lot No.	C. I. No.	Greenhouse tests		Field tests	
			First	Second	First	Second
<i>Avena sativa orientalis</i> L.:						
var. Green Mountain.....	R 110e.....	1872	1	1	1	1-
var. Long's White Tartar.....	C 1026.....		1+	1+	1+	1-
var. mutica.....	R 32i.....		1	1	2-	1
var. White Tartar <sup>a</sup> .....	R 284a.....	551	1-	1	0+	1+
Do.....	R 301a.....	1614	1-	1+	1	1
Do.....	I 98-4.....			1-		1-
Do.....	I 102j.....			1		1-
Do.....	I 102j-5.....		1+	1	2-	1-
Do.....	I 102j-7.....		1	1	1	0+
Do.....	K 6096.....		1		1-	
Do.....	C 1016.....		1+	1	1	1
Do.....	R 174a.....		1-	1	1-	1+
Do.....	C 1020.....		1+	1+		1
Do.....	W 739.....	441	1	1		0+
var. Eclipse.....	R 274a.....	843	3	3+&1-	4-	1-&4

<sup>a</sup> White Tartar is the accepted name for White Russian.

#### EXPERIMENTS WITH NINE COLLECTIONS OF RUST

At the time when this work was done (1920) physiological races of oat stem rust had not been reported, but their existence seemed probable. Experiments were undertaken in the hope of determining whether there are one or several physiological races of oat stem rust in California, and whether the oat varieties resistant to the rust collected at Berkeley are resistant to stem rust collected throughout the State.

Specimens of oat stem rust were obtained from nine oat-growing districts in California. The shaded areas of figure 1 show the approximate distribution of oat-stem rust in California based on the collections of 1919, and the numbers mark the nine localities from which the collections cultured were obtained. Stock cultures of these were kept growing, isolated from each other in cheesecloth cages, in the greenhouse. About 40 varieties of oats were chosen, including

representatives of all available species of *Avena*, and all of the varieties that proved resistant to the oat stem rust collected in Berkeley.

The experiments were made on seedlings in the greenhouse. Nine pots of seedlings of each variety were grown, one for each collection of rust. All were inoculated on the same day, placed under bell jars in the cages for 48 hours, the bell jars then removed and the plants allowed to grow in the pots. At the end of two weeks the plants were studied and rust infection recorded, and the rusted leaves packeted and pressed. On completion of the first series the entire experi-

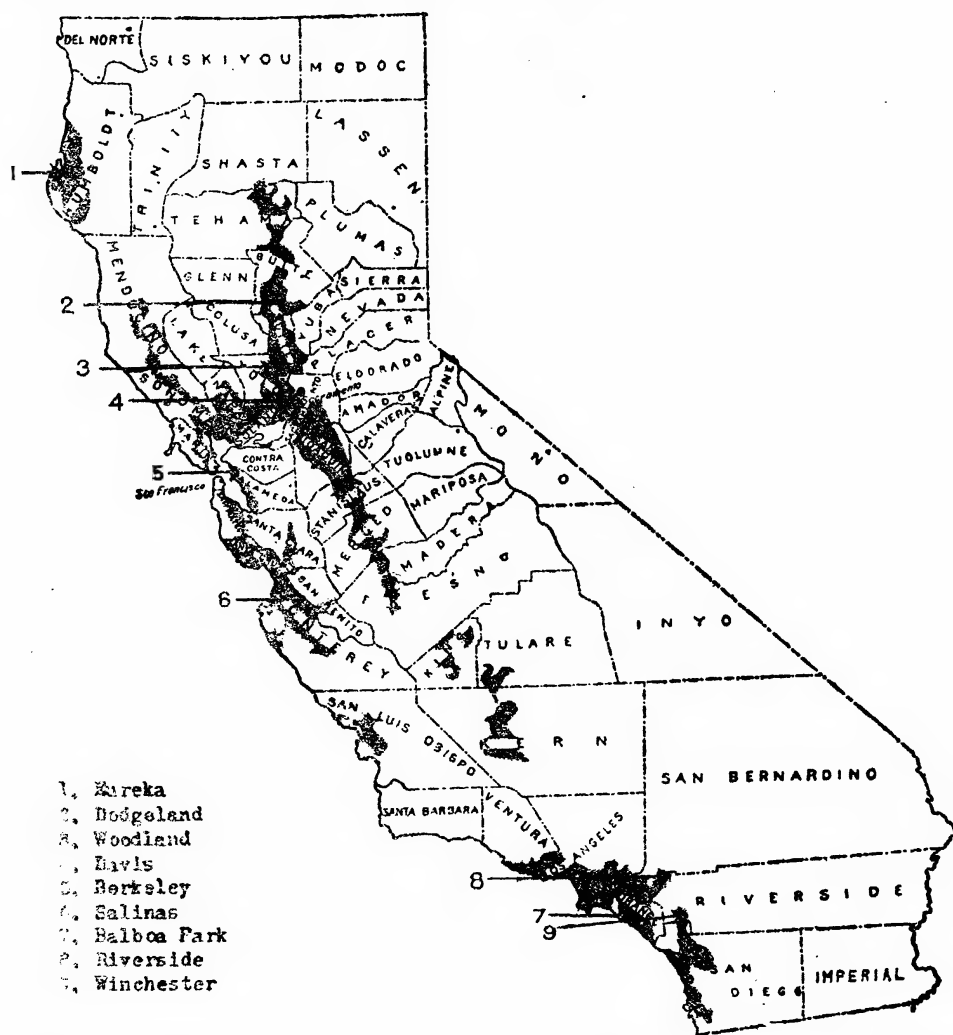


FIG. 1.—Map of California showing, by the shaded areas, the distribution of oat stem rust in California in 1919, and by numerals, the points where the nine collections of rust were made

ment was repeated. The second experiment was not completed until late in the fall of 1920, when the cooler weather and reduced light proved unfavorable for the development of the rust. These data, therefore, were discarded and this part of the work was repeated in the spring of 1921.

The results are given in Table IV and show a remarkable uniformity in the reaction of any variety of oats to the strains of rust from different localities. In general, the varieties in the first experiment that were susceptible to the rust collected at Berkeley proved equally susceptible to rust from the other sources, and the varieties resistant to the Berkeley rust proved resistant to the nine collections from other localities.

A possible exception to this statement is the reaction to the rust collected at Eureka. In general, the reaction to it was the same as to the rusts from other sources, but in the numerous lots included under White Tartar (White Russian), which is uniformly resistant to all the other rusts used (except for an occasional rogue in R 284a and I 102½), an occasional plant proved susceptible to the Eureka rust. One or two plants susceptible to the Eureka rust were found in Green Mountain, R 110e; Long's White Tartar, C 1026; White Tartar (White Russian), I 102½-7; and White Tartar (White Russian), C 1016. These occurrences are indicated by the footnote c in Table IV. It is possible that these susceptible plants also are rogues; but it seems unlikely, because the same lots of seed produced plants which reacted uniformly to the rust from other sources.

It was reported that in Eureka stem rust occurred on Richland, another resistant variety. Rust specimens were obtained from Eureka at three different times. In each case the material contained a mixture of crown and stem rusts, of which only the crown rust grew on the Richland. It may be that a form of stem rust occurs there to which Richland is susceptible, but, if so, the writers failed to obtain it. Our only indication of a different rust form is the reaction to White Tartar (White Russian), and this is inconclusive evidence.

Stakman and Levine and their associates have described in several of their papers (16, 18) a condition in which the infections on a single leaf vary greatly in size and vigor. They have named this the heterogeneous or X type of infection as noted earlier in this paper.

The oat stem rust on Joannette (R 125e) showed a similar variability and may belong to the X type. Some plants of Joannette appeared to be susceptible, some resistant, and some showed two or more distinct reactions on the same leaf. No explanation of this condition can be offered at present with any assurance.

Stakman, Levine and Bailey (17, 18) in their reports on specialization of *Puccinia graminis avenae* distinguished certainly four, and perhaps five, physiological races of stem rust. Victory (C. I. 1145), White Tartar (C. I. 1614), and Monarch Selection were used as differential hosts, with results which may be summarized roughly as follows:

Form No.	Victory	White Tartar (White Russian)	Monarch Selection
I.....	Susceptible.....	Resistant.....	Immune.
II.....	do.....	do.....	Susceptible.
III.....	do.....	Intermediate in resistance.	Do.
IV.....	do.....	Susceptible.....	Do.
V.....	.....	.....	Heterogeneous type.

So far as can be judged from the limited data available, all the collections of oat stem rust used in the experiments represent the physiological race II, as plants of Victory and Monarch Selection were susceptible and White Tartar was highly resistant.

TABLE IV.—Comparative test of oat varieties in greenhouse at Berkeley, in 1920 and 1921, with stem rust from nine localities in California

Name	Lot No.	C. I. No.	Date inoculated	Rust collected at—								
				Balboa Park	Berkeley	Davis	Dodgeland	Eureka	Riverside	Salinas	Winchester	Woodland
<i>Avena brevis</i> Roth. var.	R 134d	---	Sept. 30, 1920	3—	3	4—	3—	3—	3—	3+	3—	3+
<i>Avena fatua</i> L. var.	R 290a	1779	May 19, 1921 <sup>a</sup>	2	2+	2+	2	2+	2	3—	2	2
<i>Avena nuda</i> L.	---	---	Sept. 30, 1920	4—	4—	4—	3	3	4—	4—	3+	3
<i>var. chinensis</i>	R 30i	---	May 21, 1921	4	4	4	4	4	4	4	4	---
<i>var. tnermis</i>	R 294a	1768	Sept. 27, 1920	4	4	4	4	4	4	4	4	4
<i>Avena sativa</i> L.	---	---	May 31, 1921	4	4	4	4	4	4	4	4	---
<i>var. abyssinian</i>	R 303a	1747	Sept. 24, 1920	3—	3—	3+	3+	3—	3+	4—	3—	3—
<i>var. Black Mesdag</i>	R 117e	1877	May 19, 1921 <sup>a</sup>	4	4	4	4	4	4	4	4	---
<i>var. Black. (Received as Avena sterilis)</i>	R 295a	691	Oct. 8, 1920	4—	3+	3+	3+	3	3	4—	4—	3—
<i>var. C. I. 602 (of Etheridge)</i>	R 145a	602	May 13, 1921	3	3+	3+	3+	3+	3	3	3	3
<i>var. Culberson</i>	R 192a	273	Oct. 4, 1920	2+	3—	3—	3—	3+	2+	3	3+	3
<i>var. Garton 473. (Received as Avena sativa orientalis)</i>	R 297a	1613	Nov. 24, 1920 <sup>a</sup>	4—	4—	4—	4—	4	4	4	4	3+
<i>var. Green Russian. (Contains a resistant strain)</i>	R 276a	827	May 17, 1921	3+	3+	3—	3—	3	2+	3	3+	3+
<i>var. Green Russian</i>	I 91-4	---	Nov. 13, 1921	4— and 1+	4—	4—	4—	4	4—	4—	4—	3+
<i>var. Hay</i>	R 317a	1622	Oct. 29, 1920 <sup>a</sup>	1	1	1	1	1	1	1	1	1—
<i>var. Joaquette. (Infection may be heterogeneous or X type).</i>	R 125c	1880	Sept. 17, 1920	4— and 1+	4—	3+ and 1	3+ and 1—	3+ and 1—	3+ and 1—	3+ and 1—	3+ and 1—	3+ and 1—
<i>var. Kherson</i>	C 1021	---	Oct. 12, 1920	1	1	1	1	1	1	1	1	1
<i>var. Kherson</i>	W 1047	1198	Nov. 24, 1920 <sup>a</sup>	2+	3	3—	2—	3—	2+	2	3—	3—
<i>var. Kherson (Wisconsin Pedigree No. 156).</i>	C 1029	---	May 17, 1921 <sup>a</sup>	3—	3—	3	3	3	3	3	3	3
<i>var. Richland</i>	R 281a	787	Oct. 9, 1920	3+ and 1—	2+ and 1—	3 and 1—	1—	3 and 1—	3 and 1—	3 and 1—	3 and 1—	3+ and 1—
<i>var. Richland</i>	R 320a	787	May 21, 1921	3+ and 1—	3 and 1—	4 and 1—	4 and 1—	4 and 1—	4 and 1—	4 and 1—	4 and 1—	4 and 1—
	---	---	Oct. 4, 1920	4	4	4	4	4	4	4	4	4
	---	---	May 9, 1921	4—	4—	4—	4	4	4	4	4	4
	---	---	Oct. 9, 1920	4—	3+	4	3+	4—	3+	3+	3	3+
	---	---	May 11, 1921	4	4—	4	4	4	4	4	4	4
	---	---	Oct. 4, 1920	4	3	4	3	4	4	4	4	4
	---	---	May 9, 1921	4	3	4	3	4	4	4	3	3+
	---	---	Sept. 10, 1920	1—	1	1—	1—	1—	1—	1—	1—	1—
	---	---	Oct. 23, 1920	0+	0+	1—	1—	1—	1—	1—	1—	1—
	---	---	Sept. 20, 1920	1—	1	1—	1—	1—	1—	1—	0+	0+
	---	---	Nov. 5, 1920	0+	0+	1—	1—	1—	1—	0+	0+	0+

<sup>a</sup> The plants inoculated on these dates were affected by cold, cloudy weather, which reduced the size of the uredinia.

TABLE IV.—Comparative test of oat varieties in greenhouse at Berkeley, in 1920 and 1921, with stem rust from nine localities in California—Con.

Name	Lot No.	C. I. No.	Date inoculated	Rust collected at—									
				Balboa Park	Berkeley	Davis	Dodgeland	Eureka	Riverside	Salinas	Winchester	Woodland	
<i>Avena sativa</i> L.—Continued var. Ruakura. (An occasional susceptible plant, probably a rogue.) var. Sixty-Day Selection (of Etheridge). (Closely resembles Burt.) var. Snoma. (Received as Snoma Winter. Seed mixed, one strain susceptible, the other resistant.) var. Winter Turf.	I 73	791	Nov. 16, 1920 May 11, 1921	1 1	1+ 1-	1- 1	1- 1-	1+ 1	1 1	1- 1	0+ 1-	1- 1-	
	C 1010	1906	Oct. 13, 1920 May 9, 1921	3- 4	3+ 4	4 4	4 4	4- 4	4- 4	4- 3+	3- 4	3 4	
	C 274		Sept. 20, 1920 May 24, 1921 <sup>b</sup>	3+ and 1 1	3 and 1 1+	4 and 1+ 1+	3+ and 1+ 1+	1+ 1	4- and 1 1	4- and 1 1+	3+ and 1 1	1- 1	
	R 237a	673	Oct. 13, 1920 May 21, 1921	3- 3+	3 4	4 4	3 4	3 4	3+ 4-	3+ 4	2+ 4	3 4	
	R 274a	843	Sept. 10, 1920 May 24, 1921	3+ and 1 3+	3+ 3+	4- 3+	4- 4	4 and 1 3+	4 3+	4 3+	4 and 1 3 and 1	3+ 3	3 3
<i>Avena sativa orientalis</i> L. var. Eclipse. (Contains a resistant strain.) var. Green Mountain. (White Tartar type.) var. Long's White Tartar. var. <i>mutica</i> (White Tartar type). var. White Tartar. Do. (Occasional susceptible plant.) Do. Do. Do. (Occasional susceptible plant.) Do. Do. Do. Do. Do. (Received unnamed.) Do. (Received as Improved Snoma Winter.)	R 110e	1872	Sept. 14, 1920 Oct. 27, 1920	1 1	1 1+	1- 1	1 1	1 1	1 1	1 1	1 1	1- 1	
	C 1026		Sept. 22, 1920 May 11, 1921	1 1	1 1	2- 1	2- 1	1 1	1 1	2- 1	1 1	1 1	1- 1
	R 32i		Sept. 10, 1920 Nov. 16, 1920	1 1	1+ 1-	1+ 1	1+ 1	1 1	1 1	1 1	1 1	1 1	1- 1
	W 739	441	Sept. 17, 1920 Nov. 5, 1920	1+ 1	1+ 1	1 1	1+ 1	1+ 1	1 1	1 1	1+ 1	1 1	1- 1
	R 284a	551	Sept. 14, 1920 May 21, 1921	1+ 1	2- 1	1 1	2- 2-	1 1 <sup>c</sup>	1 1	2- 1	1+ 1	1 1	1- 2-
	R 301a	1614	Sept. 14, 1920 Oct. 27, 1920	2- 1	2- 1	1+ 1	2- 1	1 1	1 1	1+ 1	2- 1	1 1	1 1
	I 98-4		Sept. 22, 1920 Nov. 5, 1920	1+ 1	1 1	1+ 1	1+ 1	1+ 1	1 1	1 1	1 1	1 1	1- 1
	I 102 <sub>1</sub>		Sept. 20, 1920 Nov. 2, 1920	1 1	1 1	1- 1	1 1	1+ 1	1 1	1 1	1 1	1 1	1- 1
	I 102 <sub>1</sub> -5		Sept. 22, 1920 Nov. 8, 1920	1 1	2- 1	2- 1	1+ 1	2- 1	2- 1	2+ 1	1 1	1 1	1- 1
	I 102 <sub>3</sub> -7		Sept. 22, 1920 Nov. 8, 1920	1+ 1	1+ 1	2- 1	1+ 1	1+ 1 <sup>c</sup>	1 1	1 1	1+ 1	1 1	1- 1
	K 6096		Oct. 29, 1920 Sept. 17, 1920	1- 1	1- 1	1+ 1+	1- 1	1 1 <sup>e</sup>	1 1	1 1	1 1	1- 1	1- 1
	C 1016		May 9, 1921	1 1	1 1	1+ 1+	1 1	1 1	1 1	1 1	1+ 1	1 1	1+ 1





## SUMMARY

Of 217 varieties of oats grown in the greenhouse and in the field, in 1920, after being inoculated with oat stem rust collected at Berkeley, Calif., the following were found to be resistant: Richland, Ruakura I 73, Snoma, one lot of Green Russian, and all lots of White Tartar (White Russian). Eclipse and two lots of Green Russian contained a mixture of resistant and susceptible plants.

Collections of oat stem rust were made from nine oat-growing districts in California in 1920 and about 40 varieties of oats, including all previously found to be resistant, were inoculated in the greenhouse with these rusts in 1920 and again in 1921. In general, the resistant varieties proved resistant to the rust from all of these sources, and the susceptible varieties were susceptible to all.

A possible exception to this rule is the reaction of White Tartar (White Russian) to the rust from Eureka. In each of four lots of White Tartar there were one or two plants susceptible to the rust from Eureka. The same lots of White Tartar were uniformly resistant to the rusts from other parts of the State.

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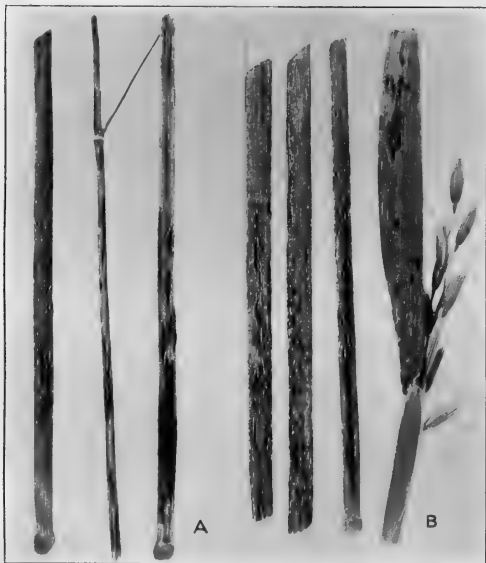
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**PLATE 1**

**Susceptible type**

- A.—Oat stem rust on Silvermine oat, I 20. Uredinia large and confluent.**  
**B.—Oat stem rust on Short Tartarian oat, C 1027.**

(720)



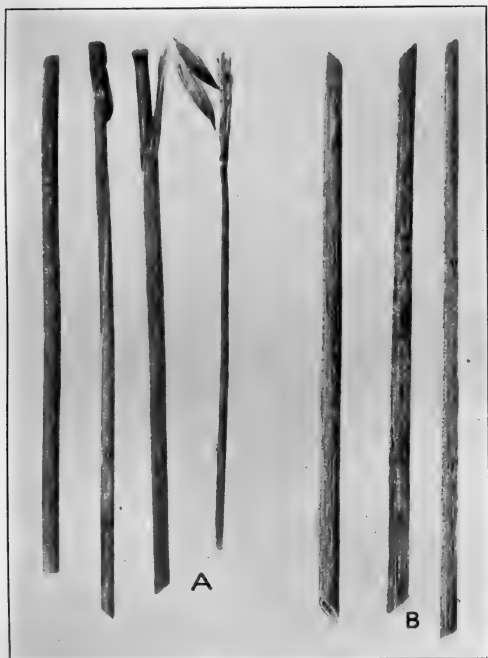


PLATE 2

Resistant type

- A.—Oat stem rust on Long's White Tartar, C 1026. Sori numerous but minute. Host but little harmed.
- B.—Similar infections on White Tartar, C 1020.

# JOURNAL OF AGRICULTURAL RESEARCH

## CONTENTS

Indicator Region	Significance of the - - - - -	Natural Vegetation of the - - - - -	Southwestern Desert - - - - -	Page
		H. L. SHANTZ and R. L. PIEMEISEL		721
Acromania, or "Crazy-Top," a Growth Disorder of Cotton	- - - - -			803
		O. F. COOK		
The Polyembryonic Development of <i>Platygaster vernalis</i>	- - - - -			829
		R. W. LEIBY and C. C. HILL		
The Inheritance of Pubescent Nodes in a Cross between Two Varieties of Wheat	-			841
		H. H. LOVE and W. T. CRAIG		

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## INDICATOR SIGNIFICANCE OF THE NATURAL VEGETATION OF THE SOUTHWESTERN DESERT REGION<sup>1</sup>

By H. L. SHANTZ, *Senior Physiologist in Charge*, and R. L. PIEMEISEL, *Assistant Physiologist, Office of Plant Physiological Investigations, Bureau of Plant Industry, United States Department of Agriculture*

The natural plant cover, if properly interpreted, indicates the crop-producing capabilities of land as well, if not better, than any series of meteorological observations or soil analyses. The value of the soil and climatic studies is not minimized by this statement, since it is only through a study of soil and meteorological conditions that the significance of the vegetation can be interpreted, especially where agriculture is not already well established.

Slight differences in either soil or climate may produce profound changes in the vegetation type, and, conversely, changes in the vegetation type may indicate only slight differences in soil or climatic conditions.

A description of the types of vegetation forms an important and necessary part of any adequate study of an agricultural region. In this paper the writers are not concerned with a descriptive or geographic study of any particular region, but with a determination of the soil and climatic conditions which are indicated by different types of natural vegetation. In other words, what are the conditions which have led to the development of the principal types of vegetation in the region or regions considered, and what do these types of vegetation signify in terms of the crop-producing capabilities of the land on which they grow?

Similar studies have been made on the Great Plains (Shantz, 1911)<sup>2</sup> and in the Great Basin (Kearney, Briggs, Shantz, McLane, and Piemeisel, 1914)<sup>3</sup> and these studies have shown that the character and types of the natural plant cover indicate the potentialities for crop production of the soil and climate which produce them.

### METHODS

The methods combine those usually employed by the systematist, the plant geographer, and the ecologist.

<sup>1</sup> Received for publication Feb. 28, 1924. The studies herein recorded have been carried on under the general direction of Dr. T. H. Kearney, Physiologist in Charge of Alkali and Drought Resistant Plant Investigations, Bureau of Plant Industry, U. S. Dept. of Agriculture. To him the authors are indebted for many suggestions in the preparation of the manuscript and for actual assistance in the field, especially in Coachella Valley, Calif., and Casa Grande, Ariz. Acknowledgment should also be made for the assistance of Homer Martin, Gardener, Alkali and Drought Resistant Plant Investigations, in the field work, and of Paul C. Standley of the United States National Herbarium, and Ivar Tidestrom, of the Bureau of Plant Industry, for the identification of the native plants listed in this paper.

<sup>2</sup> SHANTZ, H. L. NATURAL VEGETATION AS AN INDICATOR OF THE CAPABILITIES OF LAND FOR CROP PRODUCTION IN THE GREAT PLAINS AREA. U. S. Dept. Agr., Bur. Plant Indus. Bul. 201, 100 p., illus. 1911.

<sup>3</sup> KEARNEY, T. H., and others. INDICATOR SIGNIFICANCE OF VEGETATION IN TOOELE VALLEY, UTAH. Jour. Agr. Research. 1: 365-417, illus. 1914.

Herbarium material was collected whenever necessary. The plant communities have been studied in local areas and the classification applied over a wider area. The factor studies have included soil moisture determinations at the period of maximum soil moisture content and at the end of the growth period during the drought season. These soil moisture determinations have been interpreted by means of the wilting coefficient determinations on the same samples either made directly or by means of the moisture equivalent.

The soluble salt content of the soil has been determined by means of the electrical resistance method calibrated by means of gravimetric determinations, and the composition of the soil salts has been determined in each region. For climatic and weather conditions we have relied on the United States Weather

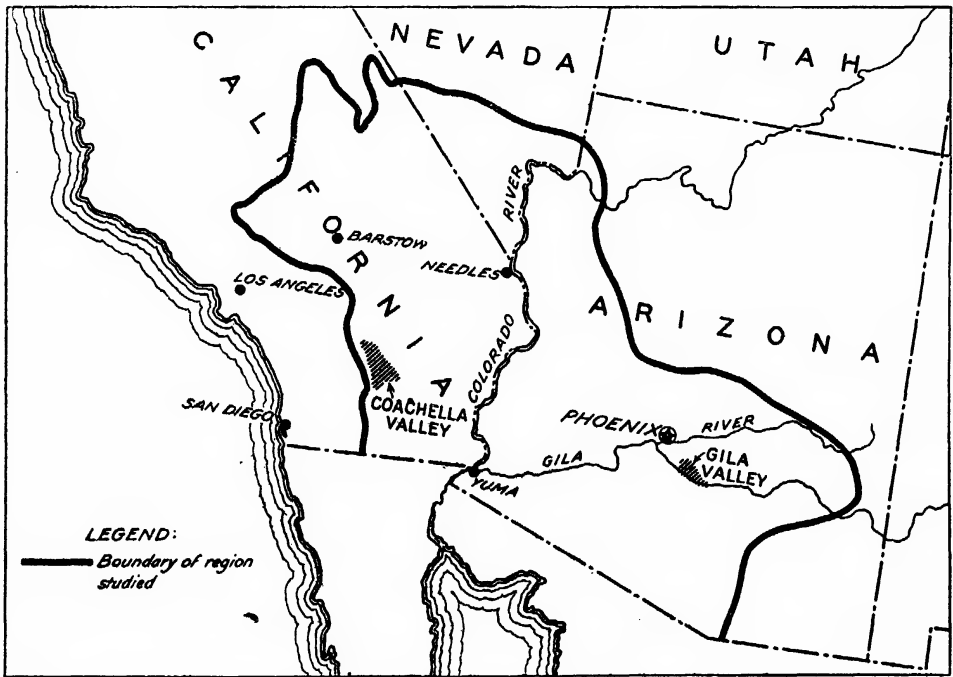


FIG. 1.—Sketch map of the southwestern desert region, showing the location of the areas in which detailed studies of the vegetation have been made. The heavy black line shows the general area studied.

Bureau records, since detached determinations continuing throughout a year or two, or only a short period, give little reliable summation of the climatic conditions or indication of the normality or abnormality of the weather conditions. When cultivated crops were grown, direct correlation was made between vegetation and crop production.

### LOCATION OF STUDIES

In this paper a study has been made of the vegetation of Coachella Valley, Calif., and of the Gila Valley in Arizona. Less detailed studies have been made at Casa Grande, Ariz.; Las Vegas and Searchlight, Nev.; Death Valley Junction, Saratoga Springs (Death Valley), Goffs, Needles, Hesperia, and the Mohave Desert, Calif.; and St. George, Utah (fig. 1). These studies were made during the years 1914, 1915, 1916, and 1917, the observations made during 1917 covering a more extended area. Similar studies have also been undertaken in New Mexico, the data from which will appear in a later publication.

## COACHELLA VALLEY

## PHYSIOGRAPHY

Coachella Valley, Calif. (fig. 1), lies between the San Bernardino Mountains on the north and east, and the San Jacinto and Santa Rosa Mountains on the south and west. The lower part of the valley bordering the Salton Sea has a very gradual slope. The upper part forms a plain that rises rapidly toward San Geronio Pass. The Coachella Valley is approximately 50 miles long, and, at the edge of the Salton Sea, 11 miles in width, the width gradually diminishing toward the north. The rise is quite abrupt on each side, making the upper zones of vegetation, creosote bush or creosote bush and bur-sage, very narrow excepting the broad alluvial fans at the mouths of all the canyons opening into the valley. In places, as at Coral Reef, these zones practically disappear. The uniformity of the slope is broken north and west of Indio, Calif., at the highest water line by a series of large sand dunes, which separate the upper higher plain from the lower and more level part of the valley.

The lower part of Coachella Valley was at one time covered by a large body of fresh water, and later probably by the sea as an extension of the Gulf of California. Full discussions are given by Blake (1858)<sup>4</sup> and Free (1914)<sup>5</sup>. The area is shown on the map by the sealevel line, and includes approximately all that part of the valley below Indio. Evidences of the former presence of water are still to be seen in the countless numbers of shells strewn over the surface of the soil of this area and also in the deposits of tufa on the rocks as at Coral Reef.

## SOILS

The soils of Coachella Valley are derived from the surrounding mountains. They are mainly of granitic origin, but some are derived from shale and sandstone.<sup>6</sup> The texture ranges from dune sand to clay loam. The two extreme types cover only small areas. The most prevalent types are a fine sandy loam and Fresno sand. The sandy loam occupies the lower part of the valley, while the Fresno sand forms the fans of alluvial soil at the mouth of canyons. The soils are generally light in color and porous. Even where the heavier soils occur on the surface there is usually a lower stratum of coarse sand or gravel. The lower part of the valley between the city of Coachella and the edge of the Salton Sea has heavy deposits of alkali. Here the water table rises high enough to keep the surface of the soil moist practically throughout the year. Alkali also occurs along the beds of some of the creeks and on the small areas of very heavy soil scattered in the upper part of the valley.

## SALINITY

The salt content of the soils in Coachella Valley varies from barely appreciable amounts to thick white crusts on the surface. The variation in the salt content and in its composition is often great in very short distances, and complicates the interpretation of the plant growth. If lands of arrowweed, pickleweed, and saltgrass are selected so that the total salt content of the soils are about the same, the arrowweed land would be preferable because of the preponderance of sulphates rather than chlorids or carbonates as is apt to be the case in the pickleweed or saltgrass land. For instance, of two excessively saline locations about 100 feet apart in the dry bed of a creek, one was covered by a rank growth of arrow-

<sup>4</sup> BLAKE, W. P. REPORT OF A GEOLOGICAL RECONNAISSANCE IN CALIFORNIA. 370 p., illus. New York. 1858.

<sup>5</sup> FREE, E. E. SKETCH OF THE GEOLOGY AND SOILS OF THE CAHUILLA BASIN. In MacDougal, D. T., The Salton Sea, p. 21-33, illus. 1914. (Carnegie Inst. Wash. Pub. 193.)

<sup>6</sup> HOLMES, J. G. SOIL SURVEY OF THE INDIO AREA, CALIFORNIA. U. S. Dept. Agr. Bur. Soils, Adv. Sheets Field Oper. 1903, Indio soil map. 1904.

weed and the other by an equally good growth of pickleweed. The analyses of the salts of the arrowweed soil showed that over 63 per cent of the total salts were sulphates (sample 9, Table I) while the pickleweed soil showed a small amount of sulphates but over 57 per cent of the total salts as chlorids (sample 13, Table I). In another place where arrowweed was dying but saltgrass was thriving the fourth foot showed in the analyses (sample 8, Table I) over 26 per cent of the total salts to be bicarbonates and 6 per cent carbonates.

## GILA VALLEY

### PHYSIOGRAPHY

The part of Arizona studied lies between Chandler and the Gila River (fig. 1) and between Florence, Casa Grande, Maricopa, and the Gila River. Detailed studies were made at Casa Grande in the fall and at Chandler both in spring and in fall. The whole of this area is a broad valley plain that slopes gradually upward toward the east and south. On the west there is an abrupt upward slope toward the Salt River Mountains.

The center of the valley has been filled in to a great depth, leaving only the tops of the mountains rising above the valley floor. The material for this extensive filling was washed down from the Salt River mountains and others higher up on the drainage course.

### SOILS

The soils of this area vary from the stony, partially disintegrated soils that lie at the bases of the mountains to the fine heavy soils, the "adobe," of the center of the valley. This change is usually gradual until the lower parts of the valley are reached, where there is often an abrupt change from the sandier types to the heavy clay loam. The nitrogen and humus content of practically all of the soils of the valley is low.<sup>7</sup>

TABLE I.—*Chemical composition of the salts of Coachella Valley soils, 1914 and 1915<sup>a</sup>*

[Crusts and surface soils were taken unless otherwise indicated. Results given in parts per 100,000 dry soil.]

Sam- ple No.	Plant growth	Car- bon- ates	Bi- car- bon- ates	Chlo- rids	Sul- phates	Cal- cium	Mag- ne- sium	So- dium	Po- tas- sium	Total al- kali
1	Cotton field.....		395	10,990	13,580	422	Trace.	13,380	Trace.	38,700
3	Cotton field.....		618	3,935	36,300	963	Trace.	18,800	Trace.	60,600
4	Washingtonia (palms).....	1,065	717	2,980	13,500	379	Trace.	9,460	Trace.	28,100
5	Allenrolfea (pickleweed).....	Trace.	149	23,550	8,210	668	Trace.	18,720	Trace.	51,300
6	Dondia (seepweed) Isocoma.....	247	545	2,430	11,820	79	Trace.	7,580	Trace.	22,700
7	Dondia (seepweed).....		233	12,700	7,100	38	Trace.	11,800	Trace.	31,900
8	Pluchea (arrowweed) dying, Distichlis (saltgrass), (fourth foot).....	17	76	26	85	17	Trace.	80	Trace.	300
9	Pluchea (arrowweed).....		180	564	10,830	239	Trace.	5,320	Trace.	17,100
10	Bare spot in field, crust.....		96	103	208	67	Trace.	127	Trace.	600
11	Bare spot in field, upper 6 inches.....		130	32	114	8	Trace.	116	Trace.	400
12	Date orchard.....		163	6,910	11,270	367	169	9,260	Trace.	28,200
13	Allenrolfea (pickleweed).....		164	4,430	374	1,090	172	1,645	Trace.	7,800
14	Mesquite (young tree).....		160	224	974	178	Trace.	468	Trace.	2,000
15	Bare spot in Pluchea (arrow- weed) and Atriplex (saltbush).....		229	8,380	5,450	6,100	392	6,725	Trace.	21,800
16	Dondia (seepweed), crust.....	Trace.	140	20,220	23,690	288	Trace.	24,170		107,69,070
17	Dondia (seepweed), depth 1 foot.....		54	1,790	3,410	620	Trace.	2,054		84,8,000
18	Pluchea (arrowweed), Atriplex (saltbush), crust.....	5,030	7,940	3,340	37,760	30		40,26,865	248	82,210

<sup>a</sup> Analyses made by the Bureau of Soils, United States Department of Agriculture.

<sup>7</sup> MEANS, T. H. SOIL SURVEY IN SALT RIVER VALLEY, ARIZONA. U. S. Dept. Agr. Bur. Soils, Rpt. Field Oper. 1900: 287-332, illus. 1900.

## SALINITY

The saline soils in the Gila Valley are confined largely to the areas where there is seepage or where the water table is high, as, for example, in the *Atriplex lentiformis* (Torr.) S. Wats. (saltbush) areas. Often considerable areas of saline land are found in the low places to which the salts have been washed by flood waters. Such are the bare flats and Dondia (seepweed) areas. Occasionally there is sufficient salt at the surface to form a crust especially in the *Atriplex lentiformis* areas. Of the salts composing the saline material, the chlorids are the most frequent and most abundant (Table II). The sulphates are next in importance. This

TABLE II.—*Chemical analyses of soils at Chandler, Arizona, 1915 a, b*

[Results given in parts per 100,000]

Plant growth	Depth of sample	Sample number	Carbonates	Bicarbonates	Chlorids	Sulphates	Calcium	Magnesium	Sodium	Potassium	Nitrates	Total solids
<i>Atriplex lentiformis</i> (saltbush).	Composite sample first, second, third, fourth feet.	9	0	83	201	74	26	0	169	-----	-----	532
<i>Atriplex lentiformis</i> .	Composite sample first, second, third, fourth feet.	10	Trace.	145	90	74	0	0	149	-----	-----	432
Bare flat.....	Composite sample first, second, third, fourth feet.	11	0	48	910	240	195	35	450	-----	40	1,990
Mesquite thicket ( <i>Prosopis glandulosa</i> ).	Composite sample first, second, third, fourth feet.	12	0	102	292	224	Trace.	0	338	-----	-----	958
<i>Atriplex polycarpa</i> (desert-sage).	Composite sample third and fourth feet.	13	0	86	360	117	37	0	279	-----	-----	892

<sup>a</sup> Analyses made by the Bureau of Soils, United States Department of Agriculture.

<sup>b</sup> See also: MEANS, T. H. SOIL SURVEY IN SALT RIVER VALLEY, ARIZONA. U. S. Dept. Agr. Bur. Soils, Rpt. Field Oper. 1900: 287-332, illus. 1900; and: ECKMANN, E. C., BALDWIN, M., and CARPENTER, E. J. SOIL SURVEY OF THE MIDDLE GILA VALLEY AREA, ARIZONA. U. S. Dept. Agr. Bur. Soils, Adv. Sheets Field Oper. 1917, 37 p., illus. 1920.

order is reversed in the Coachella Valley, where sulphates predominate. The bicarbonates, according to Table II, form a greater percentage of the salt content than in the Coachella Valley. This is also shown in an analysis by the Bureau of Soils (Table III).

The vegetation and soil conditions in the vicinity of Casa Grande, Ariz., were also studied. The salts of the soils at Casa Grande (Table IV) show more sulphates than chlorids, and in this respect resemble the salts of Coachella Valley.

## VEGETATION

## COACHELLA VALLEY

The plant communities in Coachella Valley are usually sharply differentiated, particularly those occupying the lower areas where the soil is comparatively well supplied with moisture for growth and contains large quantities of alkali salts. The uplands which are occupied by alkali-avoiding and more drought-evading species, are characterized by a more mixed vegetation. No attempt is made to distinguish the smaller plant communities, which are not as marked in the vegetation of the shrubby desert formations as they are in the grass formations. The associations, however, are much more uniform in composition and are often dominated by a single species.

TABLE III.—Chemical analyses of alkali salts of Tempe, Arizona, sheet <sup>a</sup>

Constituent	4470 Sec. 9, T. 1 N., R. 3 E. (0-12 inches)	4469 Sec. 27, T. 1 N., R. 4 E. (0-12 inches)	4541 Sec. 22, T. 1 N., R. 3 E. (crust)	4468 Sec. 3, T. 1 S., R. 4 E. (crust)	4472 Sec. 3, T. 1 S., R. 4 E. (0-12 inches)	4465 Sec. 22, T. 1 S., R. 4 E. (crust)	4466 2 miles south Kyrene (crust)
Ca.....	13.32	0.31	6.37	0.47	0.65	6.73	4.38
Mg.....	4.44	.31	4.20	.62	.57	4.03	1.24
Na.....	14.93	34.90	23.03	30.99	31.55	22.04	29.47
K.....	2.88	2.50	.00	6.40	5.01	2.12	1.65
SO <sub>4</sub> .....	5.34	12.16	26.40	9.60	9.76	.91	16.78
Cl.....	56.93	46.38	39.55	42.01	41.73	61.48	44.83
CO <sub>3</sub> .....		.18	.10	2.34	1.45		
HCO <sub>3</sub> .....	2.16	3.28		7.57	9.28	0.70	1.62
CaSO <sub>4</sub> .....	7.56	1.07	22.31	1.56	2.18	1.28	14.86
MgSO <sub>4</sub> .....		1.52	13.34	3.04	2.74		6.13
MgCl <sub>2</sub> .....	17.40		5.94			15.81	
KCl.....	5.46	4.74	0.00	12.18	9.53	4.03	3.13
NaCl.....	35.98	72.84	57.97	59.72	61.35	60.33	71.55
Na <sub>2</sub> CO <sub>3</sub> .....		.31	1.44	4.14	2.50		
NaHCO <sub>3</sub> .....	2.94	4.47		10.38	12.76	.96	2.27
Na <sub>2</sub> SO <sub>4</sub> .....		15.05		8.98	8.96		2.06
CaCl <sub>2</sub> .....	30.66					17.59	
Per cent soluble salts.....	3.33	4.47	7.24	2.56	2.48	8.58	5.80

<sup>a</sup> MEANS, T. H. SOIL SURVEY IN SALT RIVER VALLEY, ARIZONA. U. S. Dept. Agr. Bur. Soils, Rpt. Field Oper. 1900: 287-332, illus. 1900.

TABLE IV.—Chemical analyses of composite samples of second, third, and fourth feet of soil at Casa Grande, Arizona, 1915 <sup>a</sup>

[Results given in parts per 100,000]

Plant growth	Sam- ple No.	Car- bon- ates	Bi- car- bon- ates	Chlo- rides	Sul- phates	Cal- cium	Magne- sium	So- dium	Potas- sium	Ni- trates	Total solids by evap- ora- tion
Poor Covillea and Atriplex linearis.....	4	0	53	41	87	42	Trace.	50	16	Trace.	290
Atriplex polycarpa.....	14	0	55	54	166	10	Trace.	125	Trace.	Trace.	410
Atriplex fasciculata.....	5	0	36	200	249	16	Trace.	245	12	Trace.	760
Atriplex fasciculata.....	11	31	74	108	353	10	Trace.	238	Trace.	Trace.	810
Atriplex polycarpa, Atriplex linearis (in Opuntia belt).....	9	0	64	88	416	18	Trace.	255	16	Trace.	880
Atriplex linearis.....	15	6	68	122	568	41	Trace.	339	Trace.	Trace.	1,140
Dondia intermedia.....	10	106	102	114	348	10	Trace.	388	10	Trace.	1,170

<sup>a</sup> Analyses made in Dr. E. C. Shorey's laboratory, Bureau of Soils, United States Department of Agriculture.

The plant communities in Coachella Valley are as follows:

Plant communities	Dominant species
Associations—	
Yucca and cactus.....	Yucca mohavensis Sarg. (yucca). Feroactus acanthodes (Lemaire) Britton & Rose (cactus). Opuntia bigelovii Engelm. (cactus). Franseria dumosa A. Gray (bur-sage).
Creosote bush and bur-sage---	Covillea glutinosa (Engelm.) Rydb. (creosote bush) Franseria dumosa A. Gray (bur-sage).
Creosote bush.....	Covillea glutinosa (Engelm.) Rydb. (creosote bush).

## Associations—Continued.

Desert-sage.....	<i>Atriplex polycarpa</i> (Torr.) S. Wats. (desert-sage).
Seepweed.....	<i>Dondia torreyana</i> (S. Wats.) Standl. (seepweed).
Saltgrass.....	<i>Distichlis spicata</i> (L.) Greene (saltgrass).
Pickleweed.....	<i>Allenrolfea occidentalis</i> (S. Wats.) Kuntze (pickleweed).
Minor communities—	
Mesquite and chamiso.....	<i>Atriplex canescens</i> (Pursh) Nutt. (chamiso). <i>Prosopis glandulosa</i> (Torr.) (mesquite).
Washington palm.....	<i>Washingtonia filifera</i> Wendl. (Washington palm).
Arrowweed and saltbush.....	<i>Pluchea sericea</i> (Nutt.) Coville (arrowweed). <i>Atriplex lentiformis</i> (Torr.) S. Wats. (saltbush).

## DISTRIBUTION OF THE TYPES OF VEGETATION

The map (fig. 2) shows the distribution and the respective areas of the vegetation types of Coachella Valley. If the types of vegetation of the valley are divided into two groups, one including the types above and the other those below sealevel, the first group would include (a) *Yucca* and cactus; (b) creosote bush; (c) creosote bush and bur-sage; (d) mesquite and chamiso; and (e) *Washingtonia* palm, as the types above sealevel. The second group: (f) desert-sage; (g) arrowweed and saltbush; (h) seepweed; (i) saltgrass; and (j) pickleweed, the types below sealevel.

The *Yucca* and cactus area is the type of vegetation highest above sealevel. It covers the lower hills on the west side of the valley and broadens out above Whitewater over the more level but high lands.

Bordering on the lower margin is the area dominated by creosote bush. In places bur-sage is so mixed with creosote bush that it is of equal importance. The lands covered by this type are the fans at the mouths of canyons and the higher bench lands skirting the hills on each side and a broad level area across the valley between Whitewater and Palm Springs.

The mesquite and chamiso area embraces the sand dunes and sandy areas. These sandy areas occur west and southwest of Indio and also northwest beyond Indian Wells, and are the result of the winds blowing down San Geronio Pass. These winds pick up material in blowing over the higher desert lands, become laden with sand and dust and drop their burden wherever the force of the wind is slackened.

The change of the slope from the fans covered by the creosote bush to the adjoining valley floor, covered by the desert-sage, is very abrupt and sets off the two associations very definitely, as regards soil texture, soil moisture, and salt content.

While most of the lands covered by the desert-sage are below the sealevel line, some are slightly above it. They form an almost continuous belt along the sides of and at Indio, across the valley except where the sandhills or the cultivated areas break in on them.

Just below the desert-sage belt, and considerably below sealevel, are the narrow irregular strips of saline land covered with *Dondia* (seepweed). Occupying the center of the valley is the land covered with arrowweed, saltbush, or pickleweed. This is the heaviest, wettest, and most saline type of land in the valley. Small meadows formed by saltgrass are scattered within the last two types.

GILA VALLEY

The two most important types of vegetation, namely, the creosote bush and desert-sage associations, are the same as and occupy similar positions to those in the Coachella Valley. The creosote bush covers the fans and higher benches

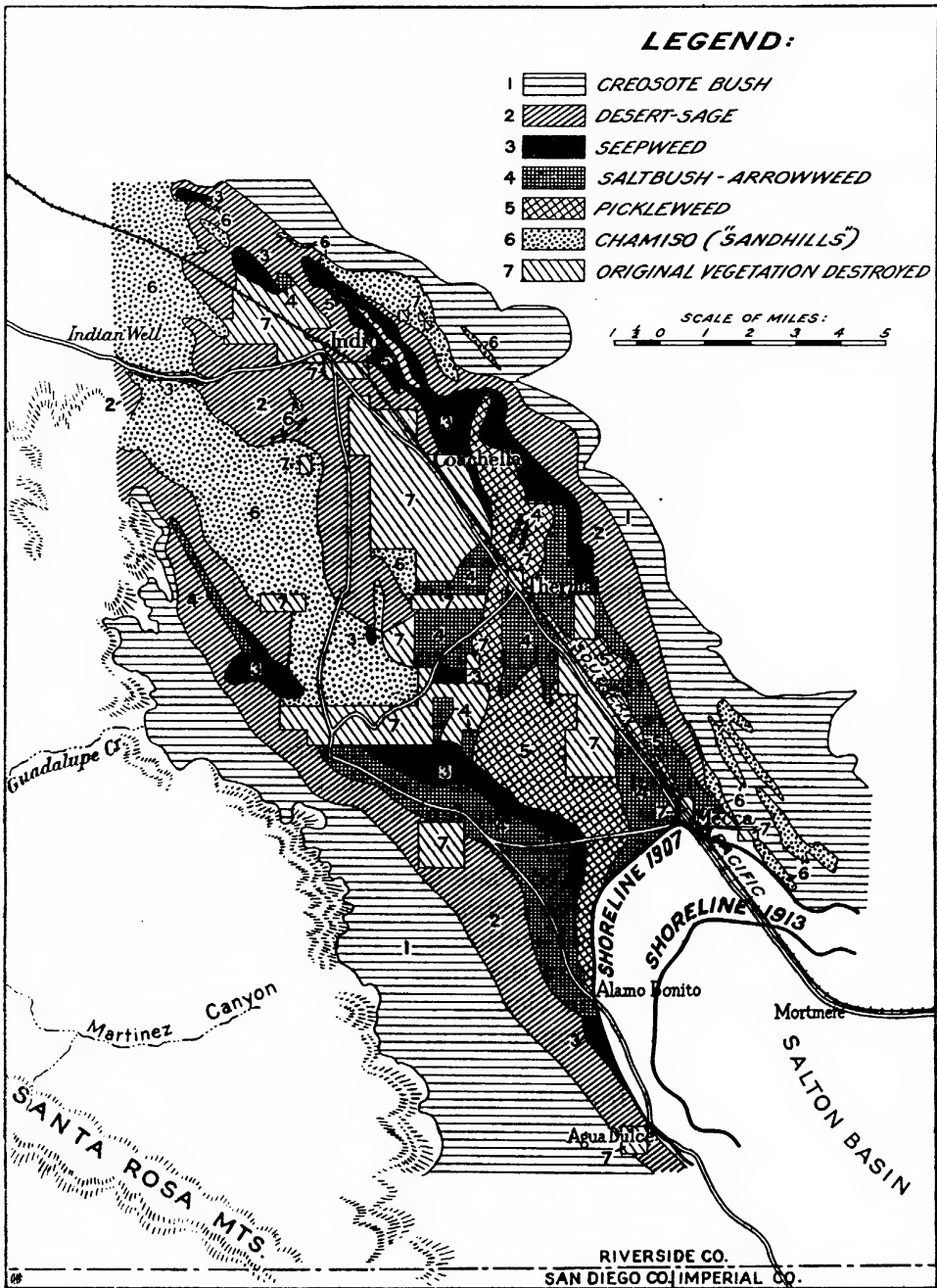


FIG. 2.—Map showing the plant communities in Coachella Valley. The map is carried only to the base of the foothills, above which there is a very irregular area of yucca and cactus. Coachella Valley, Calif., 1915.

next to the mountains and the desert-sage occupies the adjoining lower lands having a soil of finer texture with the particles more weathered. The change of slope is much more gradual than in Coachella Valley, and hence the changes in soil conditions, texture, moisture, and salt content are less abrupt. There are



many alternations of the creosote bush and desert-sage areas and the two mix to a large extent (fig. 3).

The following are the principal plant communities in the Gila Valley:

<i>Plant communities</i>	<i>Dominant species</i>
Associations—	
Giant cactus and paloverde..	<i>Carnegiea gigantea</i> (Engelm.) Britton & Rose (giant cactus). <i>Cercidium torreyanum</i> (S. Wats.) Sarg. (paloverde).
Creosote bush and bur-sage..	<i>Covillea glutinosa</i> (Engelm.) Rydb. (creosote bush). <i>Franseria dumosa</i> A. Gray (bur-sage).
Creosote bush.....	<i>Covillea glutinosa</i> (Engelm.) Rydb. (creosote bush).
Desert-sage.....	<i>Atriplex polycarpa</i> (Torr.) S. Wats. (desert-sage).
Narrowleaf saltbush.....	<i>Atriplex linearis</i> S. Wats. (narrowleaf saltbush).
Seepweed.....	<i>Dondia intermedia</i> (S. Wats.) Heller (seepweed).
Saltgrass.....	<i>Distichlis spicata</i> (L.) Greene (salt grass).
Minor communities—	
Chamiso.....	<i>Atriplex canescens</i> (Pursh) Nutt. (chamiso).
<i>Atriplex fasciculata</i> .....	<i>Atriplex fasciculata</i> S. Wats.
Saltbush.....	<i>Atriplex lentiformis</i> (Torr.) S. Wats. (saltbush).
Mesquite thicket.....	<i>Prosopis glandulosa</i> Torr. (mesquite).

The chamiso (*Atriplex canescens* (Pursh) Nutt.) covers the sandy ridges and knolls occurring in the desert-sage areas. Towards the lower parts of the valley there is a gradual change from pure desert-sage to that mixed with narrowleaf saltbush or seepweed. Still lower these shrubs mix with the mesquite. The shrubs and seepweed grow on low hummocks, composed of a light soil piled by the wind in the open spaces between the mesquite trees. In low places where there is subirrigation the mesquite becomes a thicket and excludes the other shrubs.

The lands along the rivers and creeks and the abandoned "seepland," where the water table is high, are covered with saltbush (*Atriplex lentiformis* (Torr.) S. Wats.).

In the vicinity of Casa Grande the vegetation is very similar to that in the Gila Valley. It differs most conspicuously from that in Coachella Valley in the absence of the large areas of pickleweed and arrowweed and saltbush which cover the wet saline lands. Near Casa Grande there are dry saline lands covered either with seepweed, narrowleaf saltbush or an annual *Atriplex* (*A. fasciculata* S. Wats.).

The creosote bush and desert-sage are, by far, the most important types of vegetation in this section. While they occupy the same relative position as in the Coachella and Gila Valleys the lines between them are much more obscure than in the former, and slightly more so than in the latter locality. This is due to the very gentle slope of the land, which causes the soil conditions, texture, moisture, and salinity to change very gradually. These two types of vegetation alternate frequently and near the lines of contact mix to a considerable extent.

Narrowleaf saltbush covers small tracts in the desert sage area especially where the soil is shallow. Seepweed and *Atriplex fasciculata* S. Wats. cover low flat places where the water collects after a rain and the salt content is high.

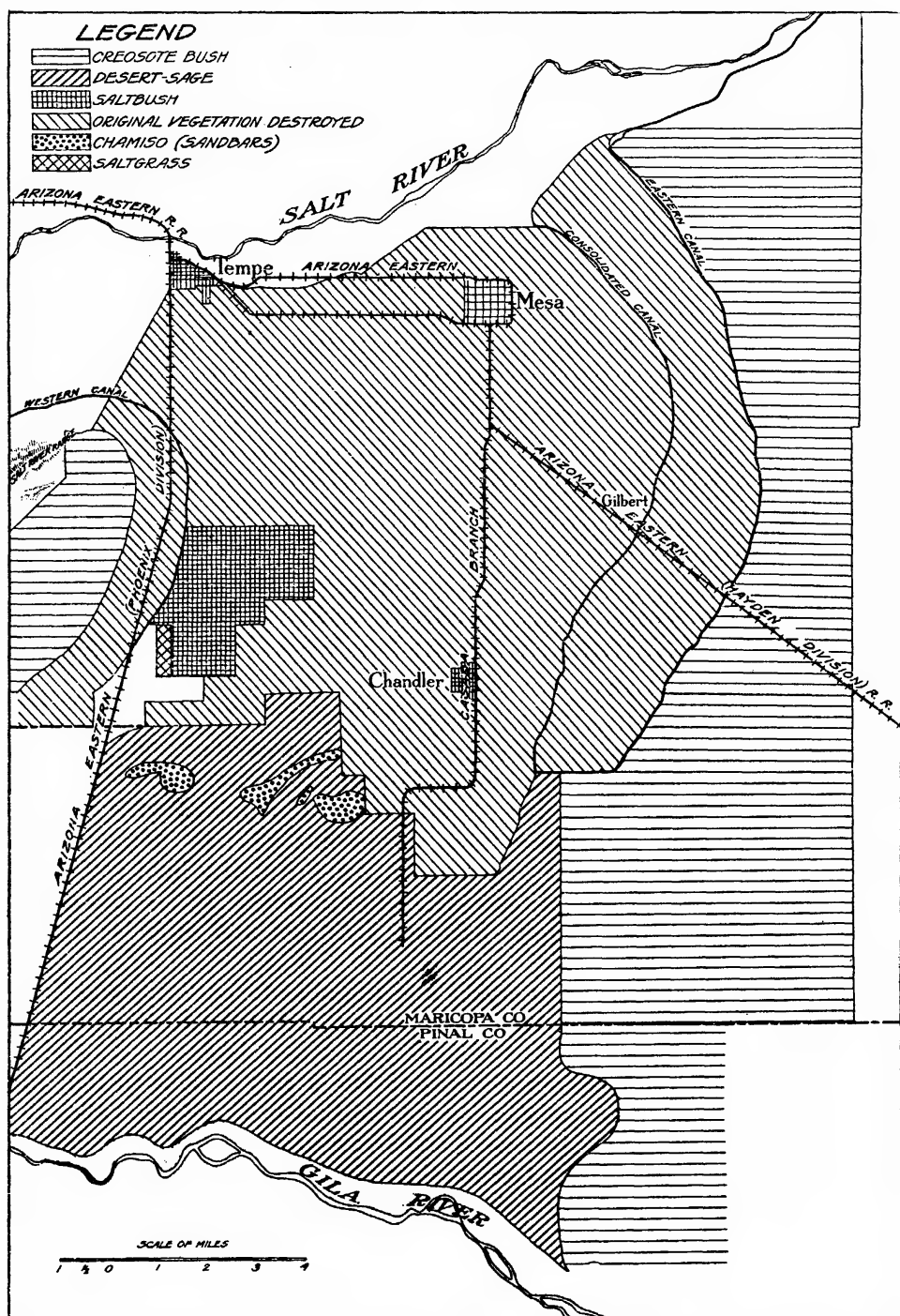


FIG. 3.—Map showing the distribution of the important plant communities in a small area in the Gila Valley. This map gives an idea of the relative importance of the different types. Gila Valley, Ariz., 1915.

### DETAILED DESCRIPTION OF PLANT COMMUNITIES

Detailed description of the plant communities together with discussions of the soil conditions found in typical areas of those communities are given in the following pages.

In so far as possible the common names of plants are used throughout the text. However, in the discussion of each association under the paragraph heading, "Botanical Composition," a list of the plants noted is given with the botanical names in full. The following list gives the common names used in the text, together with the botanical names:

Annual fescue.....	<i>Festuca octoflora</i> Walt.
Arrowweed .....	<i>Pluchea sericea</i> (Nutt.) Coville.
Bur-sage.....	<i>Franseria dumosa</i> A. Gray.
Chamiso .....	<i>Atriplex canescens</i> (Pursh) Nutt.
Creosote bush.....	<i>Covillea glutinosa</i> (Engelm.) Rydb.
Desert plantain.....	<i>Plantago erecta</i> Morris.
Desert-sage .....	<i>Atriplex polycarpa</i> (Torr.) S. Wats.
Desert-willow.....	<i>Chilopsis linearis</i> (Cav.) Sweet.
Giant cactus.....	<i>Carnegiea gigantea</i> (Engelm.) Britton & Rose.
Greasewood.....	<i>Sarcobatus vermiculatus</i> (Hook.) Torr.
Mesquite.....	<i>Prosopis glandulosa</i> Torr.
Narrowleaf saltbush.....	<i>Atriplex linearis</i> S. Wats.
Paloverde.....	<i>Cercidium torreyanum</i> (S. Wats.) Sarg.
Pickleweed .....	<i>Allenrolfea occidentalis</i> (S. Wats.) Kuntze.
Sagebrush.....	<i>Artemisia tridentata</i> Nutt.
Saltbush.....	<i>Atriplex lentiformis</i> (Torr.) S. Wats.
Saltgrass.....	<i>Distichlis spicata</i> (L.) Greene.
Seepweed.....	<i>Dondia torreyana</i> (S. Wats.) Standl. (Coachella Valley).
Do.....	<i>Dondia intermedia</i> (S. Wats.) Heller (Gila Valley).
Shadscale.....	<i>Atriplex confertifolia</i> (Torr.) S. Wats.
Washington palm.....	<i>Washingtonia filifera</i> Wendl.
Yellow pine.....	<i>Pinus ponderosa</i> Laws.

YUCCA AND CACTUS ASSOCIATION

The lower mountains surrounding the Coachella Valley are covered only with low shrubby desert growth. On the north side of the valley the hills are of clay or silt texture and weather down rapidly to form bad lands (Pl. 1, A). The vegetation on this loose cracked clay soil probably represents only successional phases, and is very sparse. It consists of the following species:

<i>Atriplex hymenelytra</i> (Torr.) S. Wats.	<i>Erigonum thomasi</i> Torr.
<i>Plantago erecta</i> Morris.	<i>Monoptilon bellioides</i> (A. Gray) Hall.
<i>Calyptridium monandrum</i> Nutt.	<i>Lotus strigosus</i> (Nutt.) Greene.
<i>Chamaesyce melanadenia</i> (Torr.) Millsp.	<i>Cryptanthus</i> sp.

At a short distance the low mountains and hills appear entirely bare, but except where bad lands occur they are characterized by the Yucca and cactus association (Pl. 1, B). The following are the more important species:

<i>Yucca mohavensis</i> Sarg.	<i>Ephedra californica</i> S. Wats.
<i>Ferocactus acanthodes</i> (Lemaire) Britton & Rose.	<i>Eriogonum fasciculatum</i> Benth.
<i>Opuntia bigelovii</i> Engelm.	<i>Krameria grayi</i> Rose & Painter.
<i>Encelia farinosa</i> A. Gray.	<i>Antirrhinum coulterianum</i> Benth.
<i>Franseria dumosa</i> A. Gray.	<i>Eriodictyon tomentosum</i> Benth.
	<i>Fouquieria splendens</i> Engelm.

Plants common along washes (Pl. 2, A) in Coachella Valley may be listed as a modification of this same association. The more important plants are:

<i>Parosela arborescens</i> (Torr.) Heller.	<i>Olneya tesota</i> A. Gray.
<i>Chilopsis linearis</i> (Cav.) Sweet.	<i>Petalonyx thurberi</i> A. Gray.
<i>Hymenoclea salsola</i> Torr. & Gray.	<i>Psathyrotes ramosissima</i> (Torr.) Gray.
<i>Cercidium torreyanum</i> (S. Wats.) Sarg.	<i>Eriogonum thomasi</i> Torr.
<i>Parosela schottii</i> (Torr.) Heller.	<i>Beloperone californica</i> Benth.
<i>Asclepias subulata</i> Decne.	<i>Lupinus</i> sp.
<i>Bebbia juncea aspera</i> Greene.	<i>Phacelia</i> sp.

#### GIANT CACTUS AND PALOVERDE ASSOCIATION

The low hills surrounding the Gila Valley and the residual soils, which are rough and stony, are characterized by the giant cactus and paloverde type of vegetation (Pl. 2, B). This is similar in general character to the Yucca and cactus type in Coachella Valley, though here the appearance is much more striking due, on the one hand, to the larger areas, a result of the gradual slope, and, on the other hand, to the giant cactus, *Carnegiea gigantea* (Engelm.) Britton & Rose, which stands high above all other plants. The higher rainfall in this region produces a much more luxuriant type of desert growth, and it is doubtful if any type of vegetation in the United States presents more interesting plants (Pl. 3, A).

This association is well developed in the region about Chandler, Ariz. The following list gives a good idea of the botanical composition:

#### PERENNIAL SPECIES

##### Common or Frequent

<i>Carnegiea gigantea</i> (Engelm.) Britton & Rose.	<i>Encelia farinosa</i> A. Gray.
<i>Cercidium torreyanum</i> (S. Wats.) Sarg.	<i>Franseria dumosa</i> A. Gray.
<i>Opuntia bigelovii</i> Engelm.	<i>Covillea glutinosa</i> (Engelm.) Rydb.
<i>Ferocactus wislizeni</i> (Engelm.) Britton & Rose.	<i>Franseria deltoides</i> Torr.
<i>Fouquieria splendens</i> Engelm.	<i>Eriogonum fasciculatum</i> Benth.
	<i>Olneya tesota</i> A. Gray.

##### Less Frequent or Rare

<i>Simmondsia californica</i> Nutt.	<i>Sphaeralcea coulteri</i> A. Gray.
-------------------------------------	--------------------------------------

#### ANNUAL AND BIENNIAL SPECIES

##### Common or Frequent

<i>Plantago fastigiata</i> Morris.	<i>Tillaeastrum aquaticum</i> (L.) Britt.
<i>Festuca octoflora</i> Walt.	<i>Phacelia</i> sp.
<i>Thelypodium lasiophyllum</i> (Hook. & Arn.) Greene.	<i>Lepidium lasiocarpum</i> Nutt.
<i>Amsinckia intermedia</i> Fisch. & Mey.	<i>Conanthus demissus</i> (A. Gray) Heller.
<i>Pectocarya penicillata</i> (Hook. & Arn.) A. DC.	<i>Baeria aristata</i> (Nutt.) Coville.
	<i>Cryptanthus</i> sp.

Less Frequent or Rare

<i>Aristida adscensionis</i> L.	<i>Lotus subpinnatus</i> Lag. (?)
<i>Brodiaea capitata pauciflora</i> Torr.	<i>Lotus strigosus</i> (Nutt.) Greene.
<i>Baeria chrysostoma</i> Fisch. & Mey.	<i>Lupinus concinnus</i> Agardb. (?)
<i>Calandrinia caulescens</i> H. B. K.	<i>Mentzelia albicaulis</i> Dougl.
<i>Chaenactis carphoclinia</i> A. Gray.	<i>Orthocarpus purpurascens</i> Benth.
<i>Chaenactis stevioides</i> Hook. & Arn.	<i>Pachylophus</i> (?)
<i>Chamaesyce polycarpa</i> (Benth.) Millsp.	<i>Phacelia crenulata</i> Torr.
<i>Cryptanthe pterocarpa</i> (Torr.) Greene.	<i>Plantago erecta</i> Morris.
<i>Monoptilon bellioides</i> (A. Gray) Hall.	<i>Salvia columbariae</i> Benth.
<i>Gilia inconspicua</i> (J. E. Smith) Dougl.	<i>Silene antirrhina</i> L.
<i>Lesquerella gordonii sessilis</i> S. Wats.	<i>Sphaerostigma decorticans</i> (Hook. & Arn.) Small.
<i>Linanthus dactylophyllus</i> (Torr.) Rydb.	
<i>Lotus</i> sp.	

The soil conditions of the giant cactus and paloverde association at Chandler, Ariz., in 1915 are shown in Table V.

TABLE V.—Soil conditions in giant cactus and paloverde association at Chandler, Ariz., 1915<sup>a</sup>

Item	Depth of soil	Date of collection				
		March 28		Average	October 5	
		Sample <sup>b</sup> No. 23	Sample <sup>c</sup> No. 25		Sample No. 23	Sample No. 25
Moisture equivalent.....	Feet 1	9.1	17.8	13.4	-----	17.6
	2	8.3	22.2	15.3	-----	21.5
	3	7.1	23.6	15.4	-----	26.5
	4	6.9	19.6	13.2	-----	19.1
Wilting coefficient.....	1	5.0	9.7	7.3	-----	9.6
	2	4.5	12.0	8.3	-----	11.7
	3	3.9	12.8	8.4	-----	14.4
	4	3.8	10.6	7.2	-----	10.4
Moisture content above or below wilting coefficient.	1	-2.1	+0.4	-0.9	-----	-3.0
	2	-0.8	+5.8	+2.5	-----	-3.7
	3	-0.6	+5.9	+2.7	-----	-6.6
	4	-0.2	+6.7	+3.3	-----	+0.5
Salt content.....	1	-----	.07	-----	-----	.11
	2	-----	.10	-----	-----	.09
	3	-----	.17	-----	-----	.14
	4	-----	.34	-----	-----	.26

<sup>a</sup> All data in this table are stated in percentages of the dry weight of the soil.  
<sup>b</sup> Taken in a typical area in the upper part of this association in a very stony soil near the hills.  
<sup>c</sup> Taken in the lower part of this association mixed with desert-sage.

CREOSOTE BUSH ASSOCIATION

TOPOGRAPHICAL RELATIONS

In the southwestern desert region the creosote bush association is the most important type of vegetation. It covers an area that includes the Mohave desert, Coachella Valley, Imperial Valley, Salt River Valley, Gila Valley, Death Valley, and most of the southern portion of New Mexico, Arizona, and the Transpecos region of western Texas.

The creosote bush areas cover the high benches, the fans at the mouth of canyons, and strips of land lying at the base of the hills. Where the slope at

the base of the hills is gentle, as in the Gila Valley, this strip of creosote bush may be several miles in width. In Coachella Valley where the slope from the hills to the valley floor is very abrupt, the creosote bush may be excluded, as at Coral Reef. In addition to the areas mentioned above, the creosote bush covers slight rises in the valley floor especially where these higher areas are composed of a light porous soil. Due to the fact that such a large portion of the creosote bush areas lies above the highest irrigation canals, these areas are not cultivated to the extent that the desert-sage lands are.

With respect to the other plant associations, the creosote bush association lies just above the desert-sage and below the giant cactus and paloverde or *Yucca* and cactus associations.

#### BOTANICAL COMPOSITION

Typical areas of creosote bush (*Covillea glutinosa* (Engelm.) Rydb.) contain practically no other shrubs (figs. 4 and 5). Most of the creosote bush land is

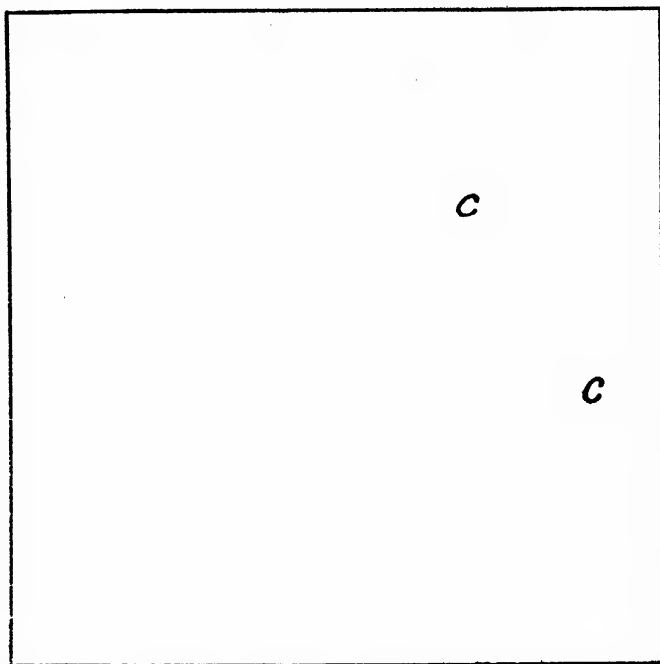


FIG. 4.—A 10-meter quadrat in a typical area in the creosote bush association. *C* indicates the individual plants of creosote bush, *Covillea glutinosa* (Engelm.) Rydb., the only shrub present. The important annuals and small perennials were *Plantago erecta* Morris, *Thelypodium lasiophyllum* (Hook. & Arn.) Greene, *Pectocarya penicillata* (Hook. & Arn.) A. DC., *Eremalche exilis* (A. Gray) Greene, *Abronia villosa* S. Wats., *Chaenactis* sp., *Chylisma cardiophylla* (Torr.) Small, *Cryptantha intermedia* (A. Gray) Greene and *Chylisma scapoidea* (Nutt.) Small. Mapped March 2, 1915, at Indian Wells, Calif.

divided by washes, the plant growth of which is very different. These washes occur very frequently on the higher slopes so that the uniformity of the creosote bush areas is interrupted. At the lower edge of the creosote bush areas where the slope is gentle these washes are much wider in extent but not so frequent, and the creosote bush areas are much more uniform. The plants occurring along the washes are merely a part of the association lying just above the creosote bush association. These plants are not included in the lists for the creosote bush areas, but are listed under the giant cactus and paloverde or *Yucca* and cactus associations.

The perennial species of plants recorded as occurring in the creosote bush areas in Coachella and Gila Valleys are given in the following lists:

## PERENNIAL SPECIES OF CREOSOTE BUSH ASSOCIATION IN COACHELLA VALLEY

## Common or Frequent

*Covillea glutinosa* (Engelm.) Rydb.  
*Franseria dumosa* A. Gray.  
*Lotus strigosus* (Nutt.) Greene.

*Abronia villosa* S. Wats.  
*Coldenia nuttallii* Hook.  
*Geraea eriocephala* (A. Gray) Blake.

## Less Frequent or Rare

*Antirrhinum coulterianum* Benth.  
*Croton californicus* Muell. Arg.  
*Eriogonum fasciculatum* Benth.  
*Gilia densifolia* Benth.  
*Hesperonia retrorsa* (Heller) Standl.  
*Hoffmanseggia microphylla* Torr.  
*Isomeris arborea* Nutt.  
*Krameria grayi* Rose & Painter.

*Nicotiana attenuata* Torr.  
*Oryzopsis hymenoides* (Roem. & Schult.)  
 Ricker.  
*Hilaria rigida* (Thurb.) Benth.  
*Aplopappus linearifolius interior* (Co-  
 ville) Jones.  
*Aplopappus linearifolius* DC.  
*Stillingia linearifolia* (Torr.) Small.

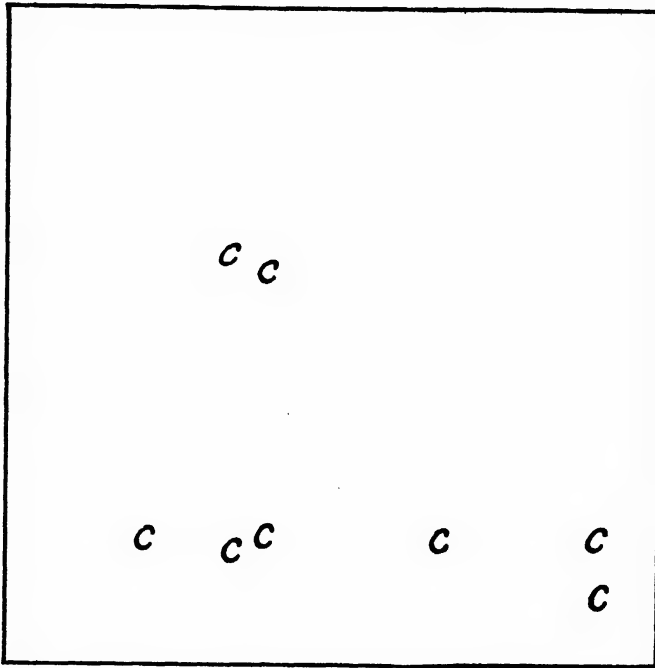


FIG. 5.—A 10-meter quadrat in a representative area of the creosote bush association, showing the individual plants of *Covillea glutinosa* (Engelm.) Rydb. indicated by C, the only shrubby species present. For a detail of the annual flora see figure 6. Mapped March 29, 1915, Chandler, Ariz.

## PERENNIAL SPECIES OF CREOSOTE BUSH ASSOCIATION IN GILA VALLEY

## Common or Frequent

*Covillea glutinosa* (Engelm.) Rydb.  
*Franseria dumosa* A. Gray.  
*Sphaeralcea* sp.

*Astragalus nuttallianus trichocarpus*  
 Torr. & Gray.

## Less Frequent or Rare

*Astragalus* sp.  
*Lycium gracilipes* A. Gray.  
*Sphaeralcea coulteri* A. Gray?

*Triodia pulchella* H. B. K.  
*Zizyphus lycioides canescens* A. Gray.

There are a large number of annuals found in the typical creosote bush areas (fig. 6.)

Of these the desert plantain (*Plantago erecta* Morris) is the most abundant and has the widest distribution. It occurs as the most important annual both in the Coachella and Gila Valleys. Annual fescue (*Festuca octoflora* Walt.) is the next

most important annual in creosote bush areas in the Gila Valley, and *Cryptanthus intermedia* (A. Gray) Greene in the Coachella Valley. Plants of *Amsinckia menziesii* (Lehm.) Nels. & Macbr. and *Thelypodium lasiophyllum* (Hook. & Arn.) Greene, tall annuals that grow clustered around the base of the creosote bush, are common to both the valleys. The other annuals or biennials recorded for the Gila and Coachella Valleys are given below:

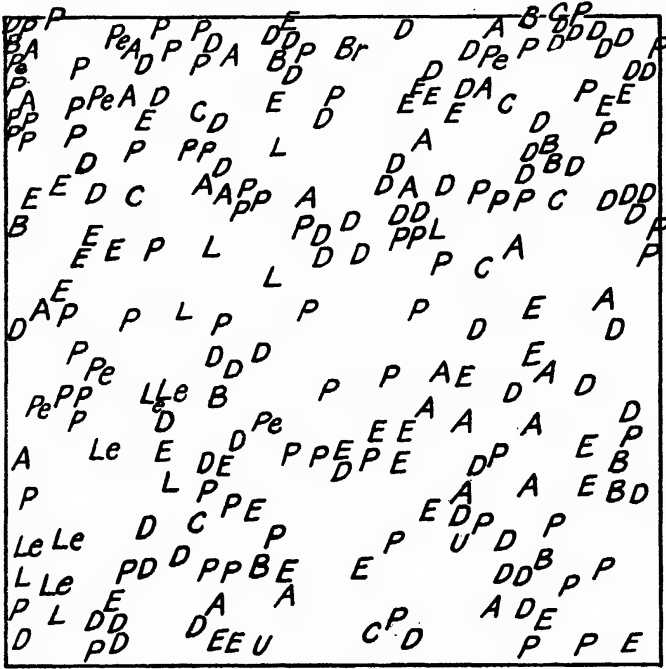


FIG. 6.—A 1-meter quadrat taken in the 10-meter quadrat, illustrated in figure 5, to show the annual flora in the creosote bush association. The letters indicate the location of each individual plant. *Plantago erecta* Morris indicated by (P) is the most important annual. The other annuals and small perennials are as follows:

- A = *Astragalus nuttallianus* [trichocarpus] Torr. & Gray  
 B = *Baeria chrysostoma* Fisch. & Mey.  
 Br = *Festuca octoflora* Walt.  
 C = *Calandrinia caulescens* H. B. K.  
 D = *Matricaria matricarioides* (Less.) Porter  
 E = *Filago arizonica* A. Gray  
 L = *Allocarya* sp.  
 Le = *Lepidium lasiocarpum* Nutt.  
 Pe = *Pectocarya penicillata* (Hook. & Arn.) A. DC.  
 U = *Daucus pusillus* Michx.

Mapped March 29, 1915, Chandler, Ariz.

#### ANNUAL AND BIENNIAL SPECIES OF THE CREOSOTE BUSH ASSOCIATION IN THE COACHELLA VALLEY

##### Common or Frequent

- |  |   |
|--|---|
| <i>Plantago erecta</i> Morris.                         | <i>Chamaesyce melanadenia</i> (Torr.) Millsp.     |
| <i>Cryptanthus intermedia</i> (A. Gray) Greene.        | <i>Lupinus concinnus</i> Agardh.                  |
| <i>Baeria chrysostoma</i> Fisch. & Mey.                | <i>Lepidium lasiocarpum</i> Nutt.                 |
| <i>Sphaerostigma veitchianum</i> (Hook.) Small.        | <i>Conanthus demissus</i> (A. Gray) Heller.       |
| <i>Chylisma scapoidea</i> (Nutt.) Small.               | <i>Eriogonum thomasti</i> Torr.                   |
| <i>Pectocarya penicillata</i> (Hook. & Arn.) A. DC.    | <i>Calyptridium monandrum</i> Nutt.               |
| <i>Thelypodium lasiophyllum</i> (Hook. & Arn.) Greene. | <i>Amsinckia menziesii</i> (Lehm.) Nels. & Macbr. |
| <i>Chylisma cardiophylla</i> (Torr.) Small.            | <i>Phacelia crenulata</i> Torr.                   |
|  | <i>Eremalche exilis</i> (A. Gray) Greene.         |
|  | <i>Mentzelia albicaulis</i> Dougl.                |
|  | <i>Dithyrea californica</i> Harv.                 |



## Less Frequent or Rare

<i>Achyronychia cooperi</i> Torr. & Gray.	<i>Euphorbia</i> sp.
<i>Anogra californica</i> (S. Wats.) Small.	<i>Hordeum murinum</i> L.
<i>Aristida adscensionis</i> L.	<i>Layia glandulosa</i> (Hook.) Hook. & Arn.
<i>Chaenactis</i> sp.	<i>Linanthus lemmoni</i> (A. Gray) Greene.
<i>Chaenactis fremontii</i> A. Gray.	<i>Lupinus</i> sp.
<i>Chaenactis carphoclinia</i> A. Gray.	<i>Mohavia viscida</i> A. Gray.
<i>Chorizanthe corrugata</i> (Torr.) Torr. & Gray.	<i>Monoptilon bellioides</i> (A. Gray) Hall.
<i>Chorizanthe rigida</i> (Torr.) Torr. & Gray.	<i>Nemoseris californica</i> (Nutt.) Greene.
<i>Coreopsis douglasii</i> (DC.) H. M. Hall.	<i>Palafoxia linearis</i> Lag.
<i>Cryptanthe muriculata</i> (A. Gray) Greene.	<i>Parosela mollis</i> (Benth.) Heller.
<i>Eriogonum thurberi</i> Torr.	<i>Phacelia</i> sp.
<i>Eriogonum inflatum</i> Torr.	<i>Phacelia distans</i> Benth.
<i>Erodium texanum</i> A. Gray.	<i>Plantago fastigiata</i> Morris.
<i>Eschscholtzia californica</i> Cham.	<i>Salvia columbariae</i> Benth.
	<i>Sphaerostigma decorticans</i> (Hook. & Arn.) Small.

## ANNUAL AND BIENNIAL SPECIES IN THE CREOSOTE BUSH ASSOCIATION IN THE GILA VALLEY

## Common or Frequent

<i>Plantago erecta</i> Morris.	<i>Matricaria matricarioides</i> (Less.) Porter.
<i>Festuca octoflora</i> Walt.	<i>Orthocarpus purpurascens</i> Benth.
<i>Lepidium lasiocarpum</i> Nutt.	<i>Erodium cicutarium</i> (L.) L'Hér.
<i>Pectocarya penicillata</i> (Hook. & Arn.) A. DC.	<i>Lesquerella gordonii sessilis</i> S. Wats.
<i>Baeria chrysostoma</i> Fisch. & Mey.	<i>Allocarya</i> sp.
<i>Amsinckia menziesii</i> (Lehm.) Nels. & Macbr.	<i>Calandrinia caulescens</i> H. B. K.
<i>Thelypodium lasiophyllum</i> (Hook. & Arn.) Greene.	<i>Lotus subpinnatus</i> Lag?
<i>Eriodium texanum</i> A. Gray.	<i>Baeria aristata</i> (Nutt.) Coville.
	<i>Filago arizonica</i> A. Gray?
	<i>Eschscholtzia</i> sp.

## Less Frequent or Rare

<i>Bowlesia septentrionalis</i> Coult. & Rose.	<i>Lupinus arizonicus</i> S. Wats.
<i>Chylisma scapoidea</i> (Nutt.) Small.	<i>Monolepis nuttalliana</i> (Schutt.) Greene.
<i>Conanthus demissus</i> (A. Gray) Heller.	<i>Pachylophus</i> ?
<i>Daucus pusillus</i> Michx.?	<i>Plagiobothrys arizonicus</i> (A. Gray) Greene.
<i>Draba cuneifolia</i> Nutt.	<i>Plantago fastigiata</i> Morris.
<i>Evax multicaulis</i> D. C.	<i>Poa bigelovii</i> Vasey & Scribn.
<i>Gaillardia arizonica</i> A. Gray.	<i>Salvia columbariae</i> Benth.
<i>Eschscholtzia californica</i> Cham.	<i>Silene antirrhina</i> L.
<i>Linaria canadensis</i> (L.) Dumort.	
<i>Lupinus</i> sp.	

## APPEARANCE

Normally the creosote bush is a dark brownish-green shrub about 5 feet high (Pl. 3, B and 4, A.) It divides at the surface of the ground into several main branches, which branch again sparingly, giving the appearance of an open bush. The dark brownish-green color is due to the thick resin-covered leaves, which are in pairs and occur most abundantly on the young twigs. In the spring these twigs bear numerous small bright yellow flowers. Pure areas of creosote bush

present a very uniform appearance. The bushes are regularly but widely spaced with the intervening ground covered with annuals. Where there is a good growth of the creosote bush covering large areas the plants are from 5 to 7 feet high, although scattered plants in very favorable locations attain a height of 10 feet or even more. A poor growth consists of low scraggy, open plants, 2 to 5 feet high, with a few branches sparsely covered with leaves. In the fall, when the moisture supply has been exhausted this growth is decidedly brown, while the good growth where moisture is still available shows dark green. The dark brownish-green of this association contrasts sharply with the light gray of the desert-sage association.

#### PHYSICAL CONDITIONS INDICATED

##### Soil Moisture

The soil in this area in Coachella Valley is light and porous and is composed of coarse unweathered particles of rock. The moisture equivalents (Table VI) show that the moisture-holding capacity is low. The penetration of water into this soil is very rapid and much deeper than in the desert-sage. In March there is available water to a depth of 4 feet, while late in summer there is none at that depth. Creosote bush is never found on soils where the water table is high.

TABLE VI.—Comparisons of spring and fall soil conditions at the same stations in typical creosote bush areas. Indio, Calif., 1915<sup>a</sup>

Item	Depth of soil	Date of collection					Average	Date of collection					Average
		Mar. 2, sample No. 6	Mar. 2, sample No. 7	Mar. 2, sample No. 8	Mar. 3, sample No. 9	Mar. 3, sample No. 10		Sept. 21, sample No. 6	Sept. 21, sample No. 7	Sept. 21, sample No. 8	Sept. 24, sample No. 9	Sept. 24, sample No. 10	
Moisture equivalent.....	Feet 1	3.8	3.4	4.2	2.3	2.6	3.3	3.7	6.5	3.6	1.9	2.8	3.7
	2	3.4	2.6	5.9	3.0	2.7	3.5	3.9	8.0	4.3	3.3	3.1	4.5
	3	3.4	2.1	2.9	3.0	2.8	2.8	4.0	5.4	3.6	3.5	3.0	3.9
	4	2.8	3.1	2.8	2.1	2.7	2.7	3.6	6.5	3.2	2.9	4.1	4.1
Wilting coefficient..	1	2.0	1.8	2.3	1.3	1.4	1.8	2.1	3.5	2.0	1.0	1.5	2.0
	2	1.8	1.4	3.2	1.6	1.5	1.9	2.1	4.4	2.3	1.8	1.7	2.5
	3	1.8	1.1	1.6	1.6	1.5	1.5	2.0	2.9	2.0	1.9	1.6	2.1
	4	1.5	1.7	1.5	1.1	1.5	1.5	2.0	3.5	1.7	1.6	2.2	2.2
Moisture content above or below wilting coefficient..	1	+1.4	+8.0	+2.8	+0.9	+1.9	+3.0	-1.1	-1.7	-1.0	-0.8	-1.2	-1.2
	2	+3.0	+8.8	+3.0	+4.4	+2.9	+4.4	-0.9	-3.2	-1.3	-1.4	-1.4	-1.6
	3	+4.3	+5.0	+3.5	+6.9	+2.4	+4.4	-1.6	-1.8	-1.3	-1.3	-1.1	-1.2
	4	+2.6	+0.9	+1.9	+3.3	+2.8	+2.3	-1.4	-1.7	-0.7	-1.2	-1.6	-1.3
Salt content <sup>b</sup> .....	1	<.01	<.01	<.01	<.01	<.01	<.01	.01	<.01	<.01	.02	<.01	.01
	2	<.01	<.01	<.01	<.01	<.01	<.01	.01	<.01	<.01	.01	<.01	.01
	3	<.01	<.01	.02	<.01	<.01	.01	.01	<.01	<.01	.01	<.01	.01
	4	.01	<.01	.02	.02	<.01	.01	.01	.02	<.01	<.01	<.01	.01

<sup>a</sup> All data in this table are stated in percentages of the dry weight of the soil.

<sup>b</sup> Electrical resistance readings over 3,000 ohms indicated less than (<) 0.01 per cent.

In considering soil moisture conditions in the different associations reference should be made to figure 7 which shows the daily rainfall for three stations in Coachella Valley. It is evident at a glance that no soil moisture had been added during the summer of 1915 and that whatever growth the plants had made was due to the soil moisture present at the beginning of the growing season in March (Table VI).

A comparison of the soil of the creosote bush land in Gila Valley with that in Coachella Valley shows (Tables VI and VII) the texture of the former to be much finer, with the moisture equivalents two or three times as high, which increases the

moisture-holding capacity of the soil but reduces the penetration. There was very little available water in the first foot when the samples were taken in the spring (March 15–27), and the amount was less in the second, third, and fourth foot than that found in Coachella Valley. The growing season was well advanced

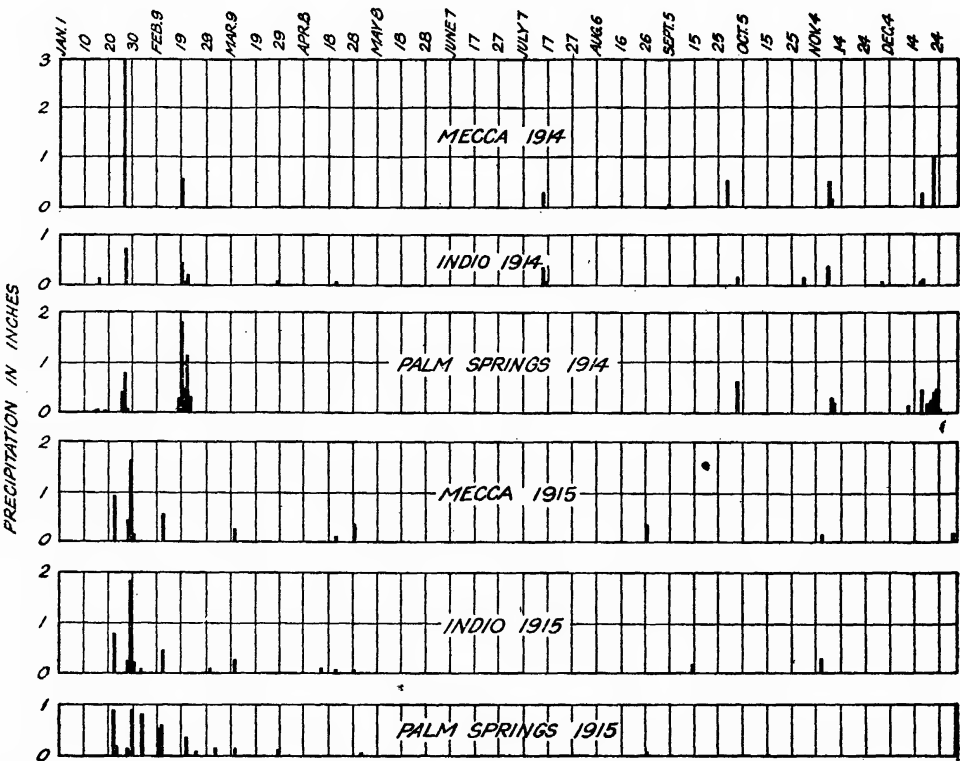


FIG. 7.—The daily rainfall in Coachella Valley, Calif., during 1914 and 1915 illustrating not only the character of the rains but the long periods of continuous droughts which occurred during the years when soil moisture studies were being made.

at that time, so that the available water had been considerably reduced. In many places the annuals had matured and were beginning to turn yellow and dry up. In comparing the available water in the creosote bush land of the two localities a difference of at least two weeks in the season of rapid growth must be taken into account. The sampling in creosote bush areas in the Coachella Valley was done March 2–3, and that in the Gila Valley March 15–27 (Tables VI and VII).

TABLE VII.—Comparison of spring and fall soil conditions at the same stations in areas of creosote bush <sup>a</sup>

Item	Depth of soil	Chandler, Ariz., 1915										Casa Grande, Ariz., 1915			
		Date of collection					Date of collection					Date of collection			
		Aver- age					Aver- age					Aver- age			
		Mar. 15, sam- ple No. 1	Mar. 20, sam- ple No. 9	Mar. 22, sam- ple No. 10	Mar. 22, sam- ple No. 11	Mar. 22, sam- ple No. 12	Mar. 27, sam- ple No. 22	Aver- age	Oct. 2, sam- ple No. 1	Oct. 6, sam- ple No. 9	Oct. 2, sam- ple No. 11	Oct. 2, sam- ple No. 12	Oct. 5, sam- ple No. 22	Oct. 11, sam- ple No. 8	Oct. 12, sam- ple No. 13
Moisture equivalent.....	Feet														
	1	10.0	7.3	10.8	10.6	10.2	9.5	9.8	11.3	13.9	10.2	12.2	9.0	9.5	9.3
	2	12.5	8.1	12.4	13.6	12.5	12.9	12.0	13.6	16.0	14.2	13.6	10.5	10.2	10.4
	3	14.8	10.4	12.9	17.0	15.2	18.2	14.8	15.4	14.7	16.0	17.1	14.4	13.9	11.2
Wilting coefficient.....	4	17.5	10.8	16.0	17.8	15.1	14.3	15.3	14.8	13.6	21.0	17.1	13.1	14.1	11.4
	1	5.4	4.0	5.9	5.8	5.5	5.2	5.3	6.1	7.6	5.5	6.6	4.9	5.2	5.1
	2	6.8	4.4	6.7	7.4	6.8	7.0	6.5	7.4	8.7	7.7	7.4	5.7	5.5	5.7
	3	8.0	5.7	7.0	9.2	8.3	9.9	8.0	8.4	8.0	8.7	9.3	7.8	7.5	6.1
Moisture content above or below wilting coefficient.	4	9.5	5.9	8.7	9.6	8.2	7.8	8.3	8.0	7.4	11.4	9.3	7.1	7.7	6.2
	1	+1.6	+0.3	+0.3	-0.6	+0.9	-1.8	+0.1	-4.4	-3.4	-3.8	-5.2	-4.4	d	d
	2	+3.1	+2.2	+2.9	+2.0	+2.5	-2.9	+1.6	-2.9	-1.4	-3.4	-3.4	-4.3	d	d
	3	+4.4	+2.1	+5.7	+2.8	+3.3	-4.3	+2.3	-3.6	-2.3	-4.7	-5.1	-5.3	d	d
Salt content.....	4	+4.2	+1.7	+5.2	+3.8	-0.4	-3.6	+1.8	-3.6	-2.4	-4.0	-4.0	-4.7	d	d
	1	.03	.02	.02	.02	.02	.02	.02	.02	.24	.06	.02	.02	.02	.02
	2	.05	.02	.02	.06	.02	.02	.03	.02	.08	.04	.02	.02	.02	.02
	3	.06	.02	.02	.06	.06	.02	.04	.07	.08	.07	.04	.02	.02	.02
Salt content.....	4	.07	.06	.06	.07	.07	.14	.08	.06	.07	.20	.07	.02	.08	.01

<sup>a</sup> All data in this table are stated in percentages of the dry weight of the soil.  
<sup>b</sup> d=dry soil.

In order to appreciate more fully the moisture conditions the reader should consult figure 8 which gives the daily rainfall in the Gila Valley during the years 1914 and 1915. The distribution of rainfall is much more uniform throughout this season than in Coachella Valley. A comparison on a monthly basis is shown in figure 9. A comparison of figures 7 and 8 with figure 9 in which the mean monthly record is shown will illustrate the great uncertainty as to quantity and distribution in these desert areas.

### Salinity

Wherever there is a good growth of the creosote bush the salt content of the soil is very low. Even a poor growth indicates a stony infertile or shallow rather

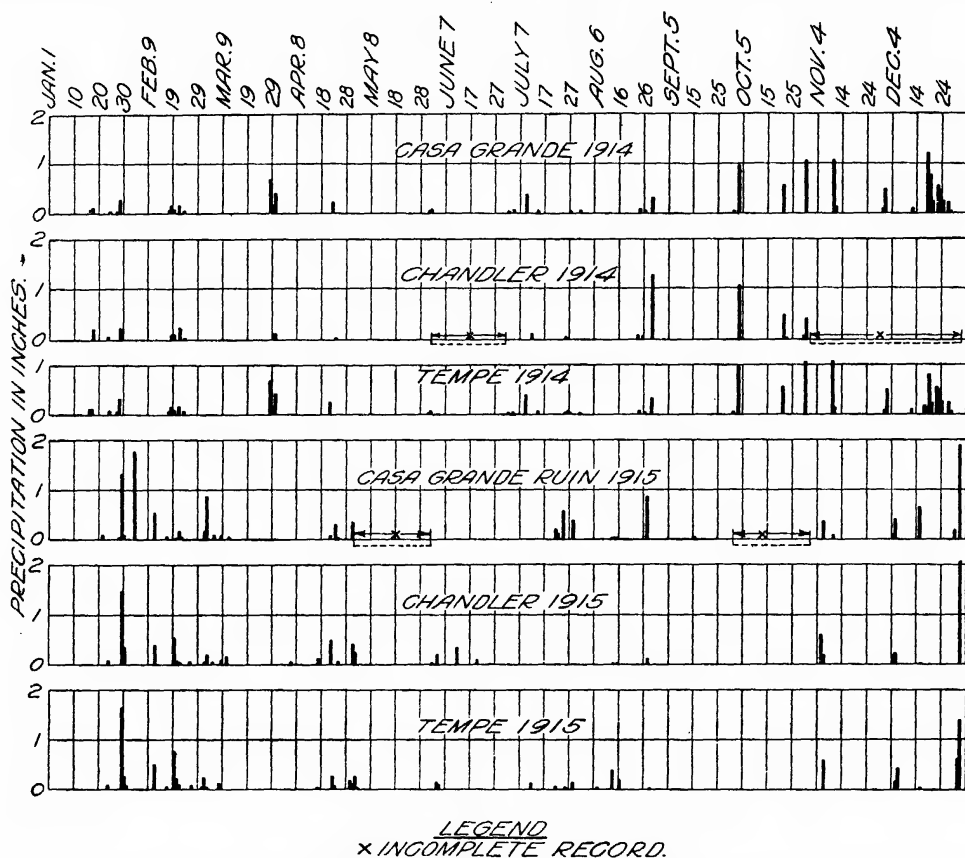


FIG. 8.—The daily rainfall in the Gila Valley, Ariz., during 1914 and 1915 illustrating not only the character of the rains but the long drought periods during the years when soil moisture studies were made.

than a saline soil (Table VIII). Individual plants of the creosote bush are sometimes found on soil containing considerable salt, but a good uniform growth has never been found on such soil. Those plants growing on saline soil are stunted and quite unlike the normal growth of the creosote bush. Comparing the creosote bush land of the two valleys (Tables VI and VII), the salt content is found to be greater in the Gila Valley. The quantity, while two or three times as great as that found in creosote bush land in Coachella Valley, is, nevertheless, small and, as far as farm crops are concerned, negligible.

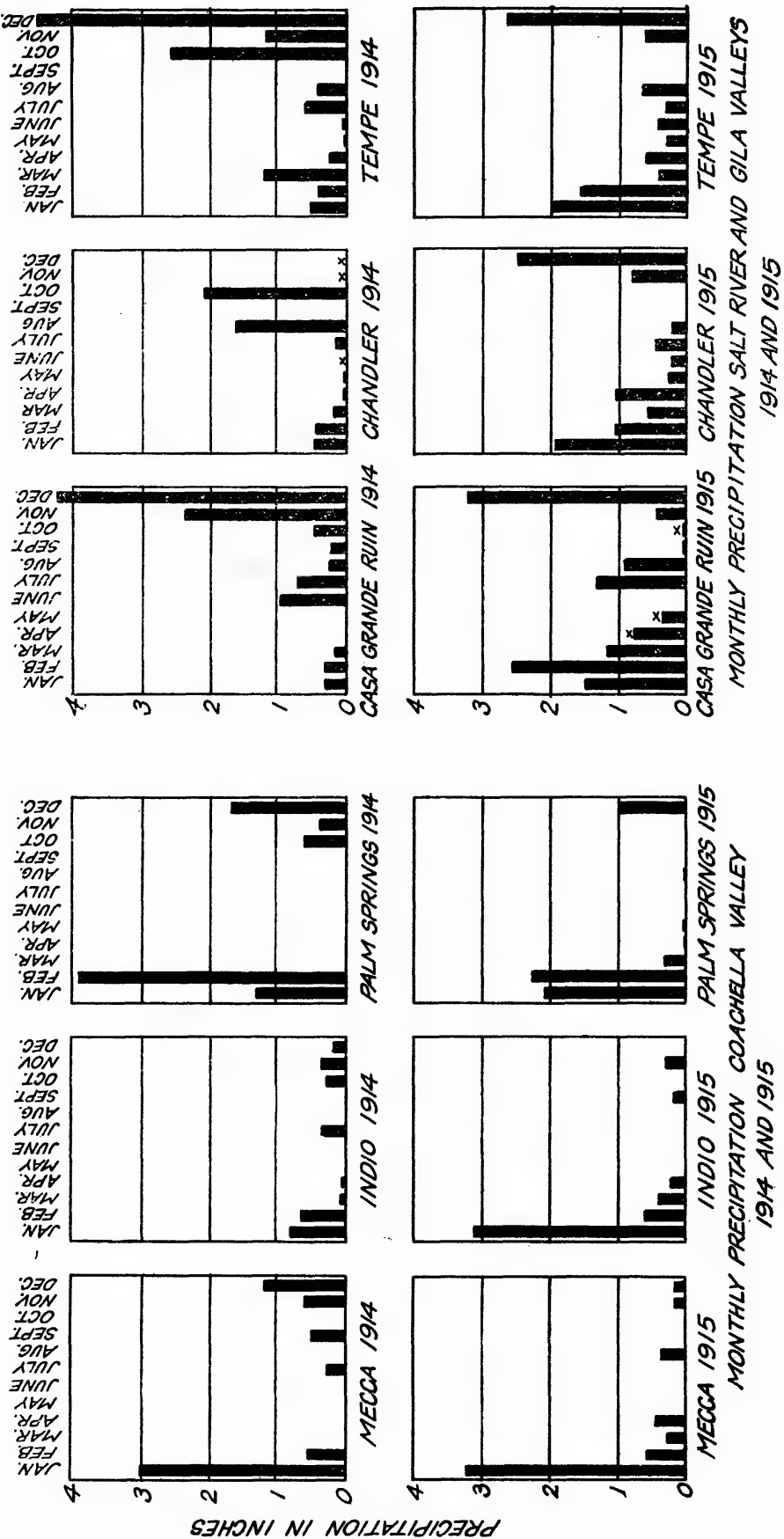


Fig. 9.—Mean monthly rainfall in the Coachella and Gila Valleys during 1914 and 1915, when studies were made of soil moisture conditions in these regions

TABLE VIII.—A comparison of soil conditions in areas of good, medium, and poor growth of creosote bush, Chandler, Ariz., 1915 <sup>a</sup>

Item	Depth of soil	Poor <sup>b</sup>			Average	Medium <sup>b</sup>		Average	Good <sup>b</sup>
		Sample No. 9	Sample No. 11	Sample No. 22		Sample No. 1	Sample No. 12		
Moisture equivalent.....	Feet 1 2 3 4	10.6 12.1 12.6 12.2	10.4 13.9 16.5 18.4	9.3 11.7 16.3 13.7	10.1, 12.5 15.1 15.2	10.7 13.1 15.1 16.2	11.2 13.1 16.2 16.1	10.9 13.1 15.6 16.2	10.8 12.4 12.9 16.0
Wilting coefficient.....	1 2 3 4	5.8 6.6 6.9 6.7	5.7 7.6 9.0 10.5	5.1 6.4 8.9 7.5	5.8 6.8 8.3 8.4	5.8 7.1 8.2 8.8	6.1 7.1 8.8 8.8	6.0 7.1 8.5 8.8	5.9 6.7 7.0 8.7
Moisture content above or below wilting coefficient.....	1 2 3 4	Spring +0.3 +2.2 +2.1 +1.7 Fall -3.4 -1.4 -2.3 -2.4	Spring -0.6 +2.0 +2.8 +3.8 Fall -3.8 -3.4 -4.7 -4.0	Spring -1.8 -2.9 -4.3 -3.6 Fall -4.4 -4.3 -5.3 -4.7	Spring -0.7 +0.5 +0.2 +0.3 Fall -3.9 -3.0 -4.1 -3.7	Spring +1.6 +3.1 +4.4 +4.2 Fall -4.4 -2.9 -3.6 -3.6	Spring +0.9 +2.5 +3.3 -0.4 Fall -5.2 -3.4 -5.1 -4.0	Spring +1.3 +2.8 +3.9 +1.9 Fall -4.8 -3.2 -4.4 -3.8	Spring +0.3 +2.9 +5.7 +5.2 Fall -----
Salt content.....	1 2 3 4	0.13 .05 .05 .07	0.04 .05 .07 .14	0.02 .02 .02 .08	0.06 .04 .05 .10	0.03 .04 .07 .07	0.02 .02 .05 .07	0.03 .03 .06 .07	0.02 .02 .02 .06

<sup>a</sup> All data given in this table are stated in percentages of the dry weight of the soil.  
<sup>b</sup> Under each of the sample numbers above (as 9, 11, 22, etc.) the averages of the spring and fall soil conditions are given, except under moisture content, where averages were not made.

### Summary of Physical Conditions

A good uniform growth of creosote bush indicates (1) at least 4 feet of light soils of coarse texture, very permeable and well drained, with a low run-off; (2) an abundant supply of available water in the upper 4 feet of soil after the winter rains, but none late in the summer; (3) a nonsaline soil to the depth of 4 feet. A poor growth of creosote bush indicates a coarse stoney infertile soil or a shallow soil having a hardpan or rocky layer within 2 or 3 feet of the surface or, more rarely, a saline subsoil.

#### ADAPTATION TO PHYSICAL CONDITIONS

In the warmer valleys, as the Coachella Valley, the growth of the creosote bush begins in February or sometimes earlier and ends before the hot summer months. During the summer and fall the plants are dormant, for by this time practically all the available soil moisture has been exhausted. The moisture in the surface foot of soil is exhausted by the large number of annuals that cover the spaces between the creosote bushes even up to the stems of the bushes themselves. The moisture below the surface foot is exhausted by the creosote bush. The creosote bush has no well-defined taproot but several almost equal branches parting near the surface of the ground (Pl. 4, B). These roots penetrate to considerable depths since the light texture of the soil and the deep penetration of the water favor a deep root system. The soil moisture here is exhausted before that of the desert-sage land and other areas in the lower parts of the valley, for the creosote bush covers the warmest slopes in the valley and therefore the annuals and the creosote bush plants start growth earlier in the spring. By May or June the annuals have matured and died while the creosote bush plants are dormant. The creosote bush lives through the long dry summer mainly because the small leathery leaves covered with a sticky resin favor a very low transpiration rate. When in a drought rest condition they become brownish and protect themselves by dropping many of the lower leaves.

#### EFFECTS OF DISTURBING FACTORS (SUCCESSIONS)

Creosote bush areas are not appreciably affected by grazing. The bushes are not browsed and the mechanical injury is slight. The herbaceous flora is, of course, greatly modified. Fires in the creosote bush areas are rare so that few opportunities have been offered for studying successions. Where the creosote bush has been cut *Franseria* probably precedes its return as a dominant plant.

#### VARIATIONS FROM THE TYPICAL ASSOCIATION

##### Creosote Bush and Bur-sage

Between the pure creosote bush areas and the foothills there is often a narrow tract occupied by a mixture of creosote bush and bur-sage (*Franseria dumosa* A. Gray) (Pl. 5, A). This mixture indicates a still lighter, coarser and very stony soil. The salt content is negligible, even less than in the typical creosote bush land. Bur-sage also occurs mixed with the creosote bush on the sandy knolls in the creosote bush areas.

##### Creosote Bush with Desert-sage

Creosote bush mixes with desert-sage (*Atriplex polycarpa* (Torr.) S. Wats.) (Pl. 5, B) near the line between these two associations. These mixed areas are very small in Coachella Valley, and indicate a higher salt content and heavier soil type than that of the typical creosote bush land, but less salt than that of



the desert-sage land (Table IX). Where the creosote bush is small, dwarfed, and scattered this is particularly true, but where the creosote bush is abundant and well developed, the salt content is negligible and the texture of the soil lighter. Where the growth of both creosote bush and desert-sage is good in this mixed area the land is among the best in this region. Its texture is somewhat lighter than typical desert-sage land, making cultivation easier and affording better drainage. The land is richer than creosote bush land and, compared to desert-sage land, there is less danger of its becoming saline. Its location makes irrigation possible where creosote bush land usually is too high.

TABLE IX.—*Comparison of spring and fall soil conditions at the same station in areas where creosote bush was mixed with desert-sage, Chandler, Ariz., 1915<sup>a</sup>*

Item	Depth of soil	Date of collection				Average	
		March 20, sample No. 8	October 5, sample No. 8	March 28, sample No. 27	October 5, sample No. 27	Spring	Fall
	<i>Feet</i>						
Moisture equivalent.....	1	6.9	6.4	10.0	11.2	8.5	8.8
	2	14.3	9.1	15.6	15.6	15.0	12.4
	3	20.0	15.7	16.0	16.8	18.0	16.3
	4	12.0	9.5	12.9	17.0	12.5	13.3
Wilting coefficient.....	1	3.8	3.5	5.4	6.1	4.6	4.8
	2	7.8	4.9	8.5	8.5	8.2	6.7
	3	10.9	8.5	8.7	9.1	9.8	8.8
	4	6.5	5.2	7.0	9.2	6.8	7.2
Moisture content above or below wilting coefficient.....	1	+1.1	-2.1	-1.3	-3.9	-0.1	-3.0
	2	+5.4	-1.4	+2.6	-3.1	+4.0	-2.3
	3	+4.8	-4.0	+0.7	-2.6	+2.8	-3.3
	4	+3.2	-2.7	-0.3	-2.8	+1.5	-2.8
Salt content.....	1	.02	.02	.06	.02	.04	.02
	2	.06	.02	.19	.12	.13	.07
	3	.27	.02	.46	.27	.37	.15
	4	.37	.06	.46	.32	.42	.19

<sup>a</sup> All data in this table are stated in percentages of the dry weight of the soil.

## DESERT-SAGE ASSOCIATION

### TOPOGRAPHICAL RELATIONS

The desert-sage association is one of the most important types of vegetation in the Southwestern deserts, but is not as wide in range as the creosote bush association. It is confined to southern Arizona and California, occurring only in the warmest valleys having a low altitude. In the Coachella and Gila Valleys it formerly covered larger areas than any other type of vegetation (figs. 2 and 3). At the present time much of the desert-sage land is under cultivation. It forms practically a continuous belt between the creosote bush area above and either the seepweed area or the saltbush and arrowweed area below. The best growth of desert-sage is found on the slightly sloping lands that are covered at certain times of the year by the flood waters from the adjoining hills. Wherever the slope of the land from foothills to valley floor is gradual, the change of soil conditions is also gradual, resulting in alternations and mixtures of the two types of vegetation. This gradual change also occurs at the lower edge of the desert-sage area where it frequently mixes with seepweed, mesquite thicket or narrowleaf saltbush.

## BOTANICAL COMPOSITION

In the typical areas of this association the desert-sage (*Atriplex polycarpa* (Torr.) S. Wats.) is the only woody species present (figs. 10 and 11) with the exception of scattered mesquite (*Prosopis glandulosa* Torr.). In the Gila Valley (fig. 11), especially in the lower places, *Lycium parviflorum* A. Gray, *Lycium gracilipes* A. Gray, *Dondia intermedia* (S. Wats.) Heller and *Atriplex linearis*

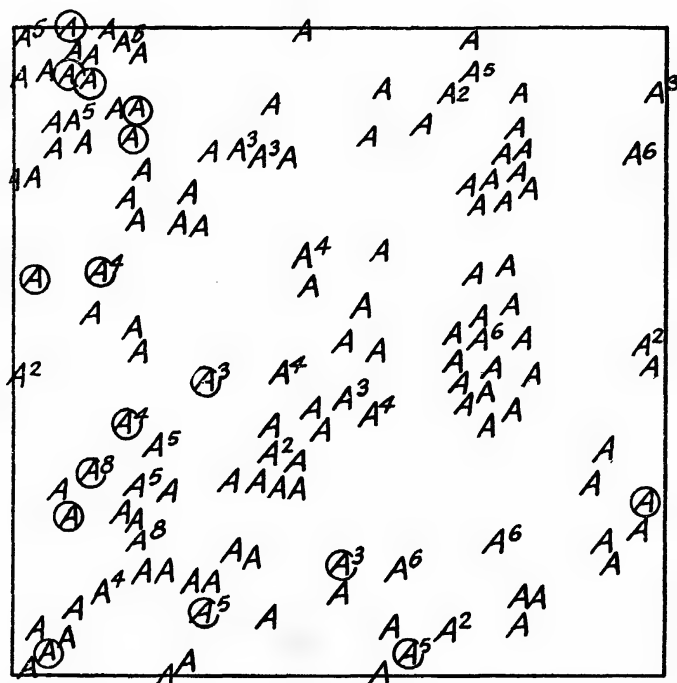


FIG. 10.—A 10-meter quadrat taken in a typical area of *Atriplex polycarpa* (Torr.) S. Wats. Individual, live plants of *Atriplex polycarpa* are indicated by A; dead plants by (A); and the number of stems to each plant by the small figures. Annuals were sparse and not blooming at this time. Mapped March 1, 1915, Indio, Calif.

S. Wats. are quite frequent. *Atriplex canescens* (Pursh) Nutt. is frequent in desert-sage areas in both valleys.

Lists containing all the perennial species noted in the desert-sage areas in Coachella and Gila Valleys are given below:

## PERENNIAL SPECIES OF THE DESERT-SAGE ASSOCIATION IN COACHELLA VALLEY

## Common or Frequent

<i>Atriplex polycarpa</i> (Torr.) S. Watts.	<i>Isocoma veneta acradenia</i> (Greene) H.
<i>Prosopis glandulosa</i> Torr.	M. Hall.
<i>Strombocarpa pubescens</i> (Benth.) A.	
Gray.	

## Less Frequent or Rare

<i>Asclepias subulata</i> Decne.	<i>Dondia torreyana</i> (S. Wats.) Standl
<i>Atriplex canescens</i> (Pursh) Nutt.	<i>Erioduction angustifolium</i> Nutt.
<i>Atriplex lentiformis</i> (Torr.) S. Wats.	<i>Pluchea sericea</i> (Nutt.) Coville.
<i>Covillea glutinosa</i> (Engelm.) Rybd.	

## PERENNIAL SPECIES OF THE DESERT-SAGE ASSOCIATION IN GILA VALLEY

## Common or Frequent

*Atriplex polycarpa* (Torr.) S. Wats.*Lycium parviflorum* A. Gray.*Lycium gracilepes* A. Gray.*Atriplex canescens* (Pursh) Nutt.*Atriplex linearis* S. Wats.*Prosopis glandulosa* Torr.

## Less Frequent or Rare

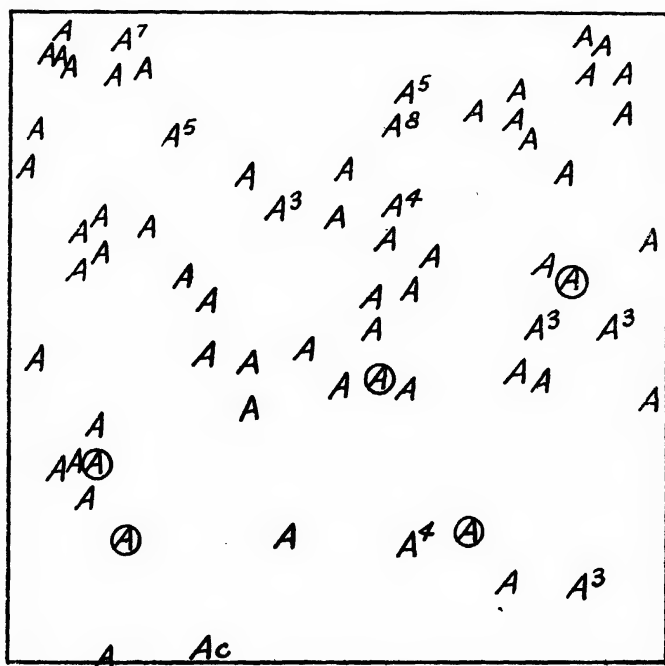
*Baccharis* sp.*Isocoma wrightii* (A. Gray) Rydb.*Sphaeralcea coulteri* A. Gray.*Sphaeralcea fendleri* A. Gray.

FIG. 11.—A 10-meter quadrat in a typical area of desert-sage, showing the location of each individual plant of *Atriplex polycarpa* (Torr.) S. Wats. indicated by A, and of *Atriplex canescens* (Pursh) Nutt., indicated by Ac, the only woody plants present. A circle around the letter indicates a dead plant. The small figures indicate the number of stems to each plant. See figure 12 for a detail of the annuals. Mapped March 29, 1915, Chandler, Ariz.

The number of annuals in the desert-sage areas as compared to that of the creosote bush or mesquite and chamiso areas is much smaller in both valleys. In Coachella Valley where flood waters frequently deposit a thin layer of silt which dries to form a crust, annuals often appeared only along the cracks in the silt. The number of annuals, both as to individuals and species, increases as the surface of the soil becomes lighter and more permeable. In the desert-sage areas *Plantago erecta* Morris is the most important annual in both valleys. In the Gila Valley *Baeria artistata* (Nutt.) Coville is also important (fig. 12).

The following lists give all the annual and biennial species noted in the desert-sage areas.

## ANNUAL AND BIENNIAL SPECIES OF THE DESERT-SAGE ASSOCIATION IN THE GILA VALLEY

## Common or Frequent

<i>Plantago erecta</i> Morris.	<i>Dipetalia subulata</i> (Webb & Berth.) Kuntze.
<i>Festuca octoflora</i> Walt.	<i>Chylisma scapoidea</i> (Nutt.) Small.
<i>Baeria chrysostoma</i> Fisch. & Mey.	<i>Eremalche exilis</i> (A. Gray) Greene.
<i>Lepidium lasiocarpum</i> Nutt.	<i>Bowlesia septentrionalis</i> Coult. & Rose.
<i>Poa bigelovii</i> Vasey & Scribn.	<i>Pectocarya penicillata</i> (Hook. & Arn.) A. DC.
<i>Thelypodium lasiophyllum</i> (Hook. & Arn.) Greene.	<i>Aster parviflorus</i> A. Gray.
<i>Baeria aristata</i> (Nutt.) Coville.	<i>Conanthus demissus</i> (A. Gray) Heller.
<i>Amsinckia menziesii</i> (Lehm.) Nels. & Macbr.	<i>Matricaria matricarioides</i> (Less.) Porter.

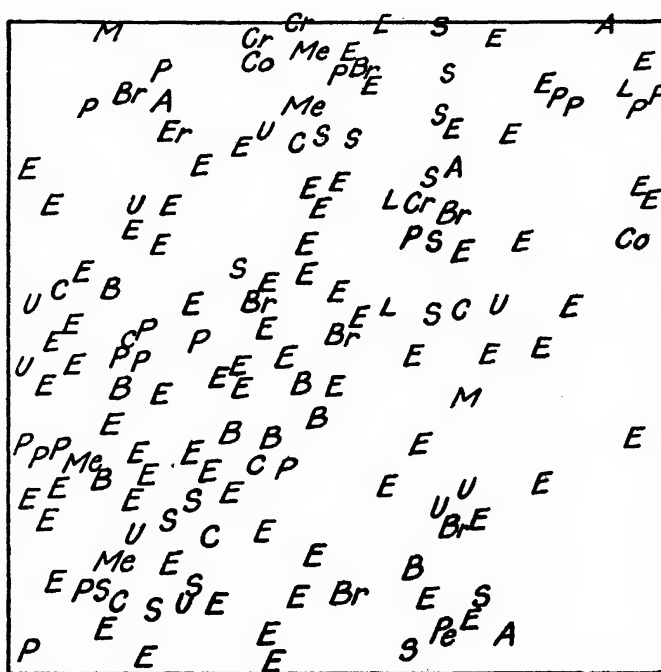


FIG. 12.—A 1-meter quadrat taken within the quadrat in figure 11 to show the annual flora of the desert-sage association. The letters give the location of each individual plant.

- A = *Aster parviflorus* A. Gray.  
 B = *Baeria chrysostoma* Fisch. & Mey.  
 Br = *Festuca octoflora* Walt.  
 C = *Chylisma scapoidea* (Nutt.) Small.  
 Co = *Conanthus demissus* (A. Gray) Heller.  
 Cr = *Thelypodium lasiophyllum* (Hook. & Arn.) Greene.  
 E = *Baeria aristata* (Nutt.) Coville.  
 L = *Lappula occidentalis* (S. Wats.) Greene.  
 M = *Eremalche exilis* (A. Gray) Greene.  
 Me = *Mentzelia albicaulis* Dougl.  
 P = *Plantago erecta* Morris.  
 Pe = *Pectocarya penicillata* (Hook. & Arn.) A. DC.  
 S = *Dipetalia subulata* (Webb. & Berth.) Kuntze.  
 U = *Spermolepis echinatus* (Nutt.) Heller.

*Amsinckia menziesii* (Lehm.) Nels. & Macbr. occurs around the desert-sage bushes.  
 Mapped March 29, 1915, Chandler, Ariz.

### Less Frequent or Rare

<i>Atriplex fasciculata</i> S. Wats.	<i>Mentzelia albicaulis</i> Dougl.
<i>Evax multicaulis</i> DC.	<i>Monolepis nuttalliana</i> (Schult.) Greene.
<i>Calyptridium monandrum</i> Nutt.	<i>Plantago fastigiata</i> Morris.
<i>Cryptanthus</i> sp.	<i>Nemoseris neomexicana</i> (A. Gray) Greene.
<i>Draba cuneifolia</i> Nutt.	<i>Senecio</i> sp.
<i>Erodium texanum</i> A. Gray.	<i>Sonchus asper</i> (L.) Hill.
<i>Erodium cicutarium</i> (L.) L'Hér.	<i>Sophia glabra</i> Woot. & Standl.
<i>Gilia floccosa</i> A. Gray.	<i>Spermolepis echinatus</i> (Nutt.) Heller.
<i>Gilia inconspicua</i> (J. E. Smith) Dougl.	<i>Microseris linearifolia</i> (DC.) A. Gray.
<i>Sphaeralcea</i> sp.	<i>Lappula occidentalis</i> (S. Wats.) Greene.
<i>Melilotus indica</i> (L.) All.	

### ANNUAL AND BIENNIAL SPECIES OF THE DESERT-SAGE ASSOCIATION IN THE COACHELLA VALLEY

#### Common or Frequent

<i>Plantago erecta</i> Morris.	<i>Sphaerostigma veitchianum</i> (Hook.) Small.
<i>Eremalche exilis</i> (A. Gray) Greene.	<i>Conanthus demissus</i> (A. Gray) Heller.
<i>Dipetalia subulata</i> (Webb & Berth.) Kuntze.	<i>Amsinckia menziesii</i> (Lehm.) Nels. & Macbr.
<i>Sophia pinnata</i> (Walt.) Howell.	
<i>Mentzelia albicaulis</i> Dougl.	

### Less Frequent or Rare

<i>Palafoxia linearis</i> Lag.	<i>Pectis papposa</i> Harv. & Gray.
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#### APPEARANCE

The gray of the desert-sage sets it off sharply from the creosote bush association even at considerable distances. A typical area shows a uniform shrubby growth (Pl. 6, B), about 3 feet high, the plants growing in clumps a few feet apart, but not nearly so widely spaced as the creosote bush. Mesquite trees are often scattered throughout this uniform growth of desert-sage. Each of the clumps is on a low hummock built up by soil that has been blown in around the bushes. The desert-sage is much smaller than the creosote bush and forms a dense rather than an open bush. There are several main branches coming up from the ground (Pl. 7, B) that are very crooked and very much branched at the top. In spring there is a dense covering of small grayish leaves on the new shoots. In fall most of the leaves have fallen off but there are large quantities of fruits.

#### PHYSICAL CONDITIONS INDICATED

##### Soil Moisture

The type of soil covered by the desert-sage is almost a fine sandy loam (Tables X and XII). The moisture-holding capacity is therefore much greater than in the case of creosote bush. Due to the flood waters in Coachella Valley the surface of the soil is crusted with a layer of silt that is not easily penetrated by the water. This intermittent depositing of silt leads to a lack of uniformity of the subsoil, and there are often alternating layers of sand and silt in the third and fourth feet. The borings made in late summer show no available water to a depth of 4 feet. The desert-sage has not been found on the moister lands where there was available moisture in all 4 feet in the late summer. Even as early as March there was rarely any available moisture in the third or fourth foot. Sometimes only the first foot was wetted by winter rains (samples 4 and 5, Table X).

TABLE X.—Comparison of spring and fall soil conditions at the same stations in typical areas of desert-sage, Indio, Calif., 1915 <sup>a</sup>

Item	Depth of soil	Date of collection					Average	Date of collection					Average
		Mar. 3, sample No. 1	Mar. 3, sample No. 2	Mar. 2, sample No. 3	Mar. 3, sample No. 4	Mar. 3, sample No. 5		Sept. 21, sample No. 1	Sept. 21, sample No. 2	Sept. 21, sample No. 3	Sept. 20, sample No. 4	Sept. 20, sample No. 5	
Moisture equivalent.	<i>Feet</i>												
	1	27.8	33.2	6.2	20.5	32.4	24.0	19.9	37.7	8.9	19.9	34.7	24.2
	2	24.9	36.5	6.4	27.4	34.4	25.9	18.0	39.2	12.3	20.7	41.5	26.3
	3	8.4	27.6	9.0	33.8	36.1	23.0	5.6	32.0	7.5	35.3	39.3	23.9
Wilting coefficient.	4	20.1	9.0	4.3	29.3	33.0	19.1	29.1	15.2	6.9	27.8	32.9	22.4
	1	15.1	18.0	3.4	11.2	17.6	13.1	10.8	20.4	4.8	10.8	18.9	13.2
	2	13.5	19.9	3.5	14.9	18.7	14.1	9.8	21.3	6.7	11.3	22.5	14.3
	3	5.0	15.0	4.9	18.4	19.6	12.6	3.0	17.4	4.1	19.2	21.4	13.0
Moisture content above or below wilting coefficient.	4	10.9	4.9	2.3	15.8	17.9	10.4	15.8	8.3	3.8	15.1	17.9	12.2
	1	-1.9	+7.0	+2.5	+6.0	+3.1	+3.3	-9.9	-18.2	-4.2	-1.7	-16.9	-10.2
	2	-1.6	+4.7	+1.1	-9.6	-9.1	-2.9	-5.3	-17.4	+0.9	-8.8	-12.1	-8.5
	3	-3.8	+0.3	-3.1	-10.2	-4.4	-4.2	-1.9	-13.6	-3.3	-11.9	-2.2	-6.6
Salt content.....	4	-7.2	-2.9	-1.4	-8.3	-5.9	-5.1	-9.5	-5.9	-1.3	-10.1	-6.2	-6.6
	1	.45	.06	-----	.06	.46	.26	.18	.06	.32	.23	.44	.25
	2	1.16	.08	-----	.06	1.48	.70	.38	.06	.14	.44	1.00	.40
	3	.32	.16	-----	.35	.43	.32	.18	.10	.06	.30	.44	.22
	4	.39	.15	-----	.23	.31	.27	.34	.13	.17	.20	.20	.21

<sup>a</sup> All data in this table are stated in percentages of the dry weight of the soil.

TABLE XI.—Soil conditions in September and October in typical areas of desert-sage, Indio, Calif., 1914<sup>a</sup>

Item	Depth of soil	Date of collection										Date of collection										Av-er-age						
		Sept. 15, sam-ple No. 15	Sept. 19, sam-ple No. 25	Sept. 22, sam-ple No. 29	Sept. 24, sam-ple No. 40	Sept. 24, sam-ple No. 41	Sept. 25, sam-ple No. 42	Sept. 25, sam-ple No. 43	Sept. 25, sam-ple No. 45	Sept. 26, sam-ple No. 46	Sept. 26, sam-ple No. 47	Sept. 26, sam-ple No. 48	Sept. 28, sam-ple No. 52	Sept. 30, sam-ple No. 53	Oct. 1, sam-ple No. 55	Oct. 6, sam-ple No. 61	Oct. 9, sam-ple No. 73	Oct. 10, sam-ple No. 74	Oct. 10, sam-ple No. 75	Oct. 13, sam-ple No. 80	Oct. 15, sam-ple No. 92		Oct. 22, sam-ple No. 95a	Oct. 22, sam-ple No. 95b	Oct. 22, sam-ple No. 95c	Oct. 26, sam-ple No. 97a	Oct. 26, sam-ple No. 97b	Oct. 26, sam-ple No. 97c
Salt content	Feet	0.40	0.17	0.12	0.19	0.16	0.02	0.06	0.12	0.18	0.51	0.17	0.13	0.78	0.20	0.18	0.05	0.18	0.02	1.40	0.23	0.22	0.20	0.80	0.64	0.12	0.54	0.30
	1	.02	.54	.34	.31	.41	.01	.06	.10	1.64	.24	.16	.13	1.10	.40	.22	.07	.22	.18	1.18	.30	1.04	.20	.56	.80	.07	1.28	.45
	2	.02	.58	.22	.37	.28	.07	.24	.16	.78	.21	.76	.09	.72	.45	.21	.18	.24	.20	.23	.26	.23	.23	.19	.49	.06	1.21	.33
	3	.17	.42	.47	.28	.19	.06	.46	.10	.32	.21	-----	.06	.34	.24	.18	.08	.15	.23	.86	.32	.75	.72	.40	.19	.23	.32	.31
Water content	1	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	-----
	2	sm	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	-----
	3	sm	sm	d	d	d	d	d	d	d	d	d	d	d	d	d	d	vsm	sm	nd	nd	d	d	d	nd	nd	nd	-----
	4	sm	sm	d	d	d	d	d	d	d	d	-----	d	d	d	d	d	vsm	sm	nd	sm	d	nd	nd	nd	nd	nd	-----
Soil type	1	fs	s	sl	sl	sl	s	sl	s	sl	sl	sl	s	sl	s	fs	fs	fs	fs	fs	sl	fs	fs	fs	sl	s	sl	-----
	2	fs	fs	s	fs	sl	s	sl	s	fs	fs	s	s	sl	s	sl	fs	sl	fs	fs	sl	fs	fs	fs	sl	s	fs	-----
	3	fs	fs	s	fs	s	s	sl	sl	fs	fs	fs	s	sl	s	sl	fs	s	sl	s	s	s	s	s	sl	s	fs	-----
	4	fs	sl	sl	fs	s	sl	sl	sl	fs	sl	-----	s	sl	s	fs	sl	s	sl	fs	sl	fs	fs	fs	s	s	fs	-----

<sup>a</sup> Salt content data in this table are stated in percentages of the dry weight of the soil. No moisture content or moisture equivalent determinations were made on the 1914 samples. Data given are field notes.

<sup>b</sup> sm=slightly moist; vsm=very slightly moist; nd=nearly dry; d=dry.

<sup>c</sup> f=fine; s=sand; l=loam.

Desert-sage in the Gila Valley indicates a soil texture (Table XII) differing very little from that of the creosote bush land, but much coarser than that of the desert-sage land in Coachella Valley (Table X). In the spring of 1915 there was available soil moisture to a depth of 4 feet. In the fall the moisture had been exhausted in the first and second feet of soil, but in some instances there was still available moisture in the third and fourth feet. This was due to the shallow root systems developed above a hard pan or heavy alkaline soil (Table XIII). That the available soil moisture was greater in spring than that in the creosote bush land was due probably to the creosote bush being on higher and warmer lands where growth begins earlier in the spring and uses the available soil moisture sooner. At the time these samples were taken, the latter part of March, the annuals on the creosote bush land were already beginning to wither and turn yellow. Those on the desert-sage land were still in their prime. At Casa Grande the soil texture of desert-sage land is much like that at Chandler, but lighter than in Coachella Valley. There is no water available in any of the 4 feet of soil in the fall.



TABLE XII.—A comparison of spring and fall soil conditions at the same stations in typical areas of desert-sage <sup>a</sup>

		Chandler, Ariz., 1915										Casa Grande, Ariz., 1915												
Item	Depth of soil	Date of collection								Aver- age	Date of collection								Date of collection					
		Date of collection									Date of collection								Date of collection					
		Mar. 16, sam-ple No. 3	Mar. 20, sam-ple No. 5	Mar. 20, sam-ple No. 6	Mar. 20, sam-ple No. 7	Mar. 25, sam-ple No. 16	Mar. 25, sam-ple No. 17	Mar. 26, sam-ple No. 19	Mar. 28, sam-ple No. 26		Oct. 3, sam-ple No. 3	Oct. 5, sam-ple No. 5	Oct. 5, sam-ple No. 6	Oct. 5, sam-ple No. 7	Oct. 1, sam-ple No. 16	Oct. 1, sam-ple No. 17	Oct. 6, sam-ple No. 19	Oct. 5, sam-ple No. 26	Oct. 11, sam-ple No. 1	Oct. 11, sam-ple No. 7	Oct. 12, sam-ple No. 14	Aver- age		
Moisture equivalent.....	Feet 1 2 3 4	9.9 13.9 17.3 19.7	16.8 17.9 15.2 12.9	19.8 11.9 11.7 13.9	16.8 18.4 22.0 15.4	11.6 18.1 16.9 15.0	6.1 6.9 8.5 8.2	8.2 8.6 10.2 11.6	8.2 21.7 21.9 19.2	11.5 21.7 21.9 19.2	12.6 14.7 15.5 14.5	8.7 11.5 19.3 20.9	14.9 17.4 13.3 14.0	18.5 10.8 11.0 12.6	13.0 21.4 21.3 14.2	10.5 16.5 18.8 27.2	6.8 7.6 8.2 8.4	8.7 9.4 11.8 13.9	12.9 21.6 21.9 18.9	11.7 14.5 13.7 16.3	10.4 13.7 13.5 18.7	10.2 13.5 14.5 11.9	9.1 12.8 10.9 10.7	9.9 13.3 15.0 13.8
Wilting coefficient.....	1 2 3 4	5.4 7.6 9.4 10.7	9.1 9.7 8.3 7.0	10.8 6.5 6.4 7.5	9.1 10.0 12.0 8.4	6.3 9.8 9.2 8.2	3.3 3.8 4.6 4.5	4.5 4.7 5.5 6.3	6.2 11.8 11.9 10.4	6.8 8.0 8.4 7.9	6.8 8.0 8.4 7.9	4.7 6.2 10.5 11.4	8.1 9.5 7.2 7.6	10.1 5.9 6.0 6.9	7.1 11.6 11.6 7.7	5.7 8.9 10.2 14.9	3.7 4.1 4.5 4.6	4.7 5.1 6.4 7.6	7.0 11.7 11.9 10.3	6.4 7.9 8.5 8.9	5.7 7.4 10.6 10.2	5.5 7.3 7.9 6.5	5.0 7.0 5.9 5.8	5.4 7.2 8.1 7.5
Moisture content above or below wilting coefficient.	1 2 3 4	+2.8 +6.5 +6.4 +8.1	+4.0 +6.5 +4.5 +4.0	+0.9 +2.7 +1.4 -3.2	+2.8 +2.6 -2.4 -2.9	-0.8 +1.9 +4.8 +9.7	+0.3 +2.3 +2.4 +3.7	-0.6 +0.9 +1.5 +2.6	-1.2 +4.2 +3.2 +1.6	+1.0 +3.5 +2.7 +3.0	-1.2 +4.2 +3.2 +1.6	-3.2 +0.9 +2.9 +3.7	-5.7 -3.9 -1.5 -2.5	-8.2 -3.1 -2.6 -3.4	-4.6 -4.0 -1.9 -2.4	-3.3 -1.7 +3.5 +7.7	-2.3 -0.3 +2.5 +3.6	-3.5 -2.1 -2.9 -2.3	-4.4 -2.1 +0.1 +0.4	b d d d d	d d d d	d d d d	d d d d	-----
Salt content.....	1 2 3 4	.06 .46 .04 1.06	.05 .06 .05 .06	.02 .06 .07 .02	.10 .80 .44 .44	.06 .10 .38 .94	.02 .03 .27 .44	.01 .02 .02 .06	.02 .14 .63 .86	.04 .21 .35 .49	.04 .21 .35 .49	.02 .19 .51 .21	.06 .04 .02 .02	.12 .06 .02 .02	.02 .31 .37 .82	.16 .34 .62 .82	.11 .35 .41 .62	.01 .01 .04 .15	.14 .59 .72 .83	.08 .25 .34 .35	.02 .02 .04 .04	.07 .17 .22 .19	.04 .07 .08 .14	

<sup>a</sup> All data in this table are stated in percentages of the dry weight of the soil.

b d=dry soil.

TABLE XIII.—A comparison of soil conditions in areas of good, medium, and poor growth of desert-sage, Chandler, Ariz., 1915<sup>a</sup>

Item	Depth of soil	Poor <sup>b</sup>			Mean	Medium <sup>b</sup>		Mean	Good <sup>b</sup>			Mean
		Sample No. 3	Sample No. 7	Sample No. 26		Sample No. 16	Sample No. 17		Sample No. 5	Sample No. 6	Sample No. 19	
Moisture equivalent ----	Feet											
	1	9.3	14.9	12.2	12.1	11.1	6.5	8.8	15.9	19.2	8.5	14.5
	2	12.7	19.9	21.7	18.1	17.3	7.3	12.3	17.7	11.4	9.0	12.7
	3	18.3	21.7	21.9	20.6	17.9	8.4	13.2	14.3	11.4	11.0	12.2
Wilting coefficient ----	4	20.3	14.8	19.1	18.1	21.1	8.3	14.7	13.5	13.3	12.8	13.2
	1	5.1	8.1	6.6	6.6	6.0	3.5	4.8	8.6	10.5	4.6	7.9
	2	6.9	10.8	11.8	9.8	9.4	4.0	6.7	9.6	6.2	4.9	6.9
	3	10.0	11.8	11.9	11.2	9.7	4.6	7.2	7.8	7.2	6.0	6.7
Moisture content above or below wilting coefficient.	4	11.1	8.1	10.4	9.9	11.6	4.6	8.1	7.3	7.2	7.0	7.2
	Spring	+2.8	+2.8	-1.2	+1.5	-0.8	+0.3	-0.3	+4.0	+0.9	-0.6	+1.4
	Fall	+3.2	-4.6	-4.3	-4.0	-3.3	-2.3	-2.8	-5.7	-8.2	-3.5	-5.8
	Spring	+6.5	+4.0	-2.1	+4.4	-1.7	+2.3	+2.1	+6.5	+2.7	+0.9	+3.4
Salt content ----	2	+0.9	-2.6	-2.1	-1.7	-1.9	-0.3	-1.0	-3.9	-3.1	-1.8	-2.9
	3	+2.9	-2.4	+0.8	+2.4	+3.5	+2.5	+3.6	+4.5	+1.4	+1.5	+2.5
	4	+8.1	-2.9	+1.6	+2.3	+9.7	+3.7	+6.0	+4.0	-3.2	+2.6	+1.1
	Fall	+3.7	-2.4	-1.4	0.0	+7.7	+4.6	+6.2	-2.5	-3.4	-2.3	-2.7
Field notes on hardpan, gravel, lime, etc.	1	0.04	0.06	0.08	0.06	0.11	0.07	0.09	0.06	0.07	0.01	0.05
	2	.33	.56	.37	.42	.22	.19	.21	.03	.06	.02	.04
	3	.73	.41	.68	.50	.34	.61	.42	.05	.03	.04	.04
	4	.64	.38	.85	.62	.88	.43	.66	.04	.02	.11	.06
Veins of white salt.	1	Sandy	Lime and hardpan.	Lime and hardpan.								
	2	Heavy soil.	Lime and hardpan.	Lime and hardpan.								
	3	Veins of white salt.										
	4	do										

<sup>a</sup> All data in this table are stated in percentages of the dry weight of the soil.<sup>b</sup> Averages of spring and fall figures were made except under moisture content.

### Salinity

The salt content of desert-sage land is shown in Tables X, XI, and XII. All of the 4 feet of soil show appreciable quantities of salt, and vary considerably. This is more easily understood if the fact is considered that pure areas of desert-sage may be either of a good growth, 3 to 4 feet high, or poor scrubby growth, 1 to 2 feet high. Samples taken in these two extremes of the plant growth in Coachella Valley show considerable difference in the salt content (Table XIV). A typical desert-sage area of a good growth indicates land having a negligible amount of salt. The average amount of salt of all the samples in the desert-sage is much higher than a similar average of the creosote bush samples (Table XXXIV). The abrupt changes in the salinity as well as the texture and moisture of the soil follow closely the abrupt changes of the elevation and slope of the land covered by these two associations.

The desert-sage in the Gila Valley indicates a lower salt content in the first two feet of soil than in the Coachella Valley. With the exception of the first foot, the salt content is four times as great as in the creosote bush land. On the whole, a typical area of desert-sage indicates land that is not too saline for farming purposes. The salt content of the soil varies with the growth and conditions of the plants forming the area. If, as in the case of the Coachella Valley data the data in Table XII is separated into three groups, according to the field notes on the growth and conditions of the plants, whether poor, medium, or good (Table XIII) it will be seen that the salt content is much less in the good group while it rises considerably, especially in the third and fourth feet, in the poor group.

The salt content is lower in desert-sage land in the Gila Valley, somewhat higher in the Coachella Valley, and negligible around Casa Grande where it has approximately the same salt content as the creosote bush land of the Gila Valley.

### Summary of Physical Conditions

The desert-sage indicates land of a much finer texture and greater moisture-holding capacity than the creosote bush land. The penetration of the water, however, is much less on the desert-sage land, due to the heavier soil and layer of silt so often deposited on the surface as a result of flood water following the winter rains. A good growth of desert-sage in the Gila Valley indicates land that has a soil texture ranging from a sandy to a fine sandy loam, and somewhat coarser than that indicated by the same plant in the Coachella Valley. There is available moisture to a depth of 4 feet in spring, but none in the fall. The amount of available water is therefore much less than on the creosote bush land. The salt content is much higher in the desert-sage land though, wherever there is a good growth of desert sage, the salt content of the soil is negligible. Where the growth is poor the salt content is greater, but never excessive.

TABLE XIV.—A comparison of soil conditions in areas of good, medium, and poor growth of desert-sage, Indio, Calif., 1915 <sup>a</sup>

Item	Depth of soil	Poor	Medium		Average	Good		Average
		Sample No. 1	Sample No. 4	Sample No. 5		Sample No. 2	Sample No. 5	
Moisture equivalent.....	Feet							
	1	23.9	20.2	33.6	26.9	35.5	7.6	21.6
	2	21.5	24.1	38.0	31.1	37.9	9.4	23.7
	3	7.0	34.6	37.7	36.2	29.8	8.3	19.1
Wilting coefficient.....	4	24.6	28.6	33.0	30.8	12.1	3.6	8.9
	1	13.0	11.0	18.3	14.7	19.2	4.1	11.7
	2	11.7	13.1	20.6	16.9	20.6	5.1	12.9
	3	4.0	18.8	20.5	19.7	16.2	4.5	10.4
Moisture content above or below wilting coefficient.....	4	13.4	15.5	17.9	16.7	6.6	3.1	4.9
	Spring	Spring	Spring	Spring	Spring	Spring	Spring	Spring
	Fall	Fall	Fall	Fall	Fall	Fall	Fall	Fall
Salt content.....	1	0.32	0.15	0.45	0.30	0.06	0.32	0.19
	2	.77	.25	1.24	.75	.07	.14	.11
	3	.25	.33	.44	.39	.13	.06	.10
	4	.37	.22	.26	.24	.14	.17	.16

<sup>a</sup> All data in this table are stated in percentages of the dry weight of the soil. Averages of the spring and fall data were made except under moisture content.

## ADAPTIONS TO PHYSICAL CONDITIONS

The desert-sage has a well developed tap root besides numerous supplementary roots just below the surface of the ground (Pl. 7, B). Plants of good growth feed on at least 3 to 4 feet of soil, but those of poor growth, where the penetration of water is less, do not feed below 2 feet. In general, the period of growth of the desert-sage corresponds to that of the creosote bush but since the areas covered by the former are not so favorably situated with regard to temperature, the beginning of the season's growth of the desert-sage lags about two weeks behind that of the creosote bush. This difference is reflected in the growth of the annuals. In 1915 in the middle of February annuals were in bloom in the creosote-bush areas, but were not blooming up to March 1 in the desert-sage areas. During the summer months the leaves are shed until by fall the plant is bare except for the fruits, which, in favorable years, are so heavy that when they have fallen they cover the ground. The desert-sage has to resist the most severe drought conditions of any of the plant associations in Coachella Valley. The areas having the poorest growth were found next to the seepweed, or, in the Gila Valley, near narrowleaf saltbush where the salt content is high, especially below the surface foot. The areas of the best growth were those at the upper end of the desert-sage belt bordering the sand hills or the creosote bush areas.

The number of annuals on the desert sage land is very small compared to that of the creosote bush land and therefore the moisture of the surface foot is not exhausted by them as in the case of the creosote bush land. As there are depressions between the desert-sage bushes where the water accumulates after rains, the plants avail themselves of this surface moisture by means of the superficial part of their root systems.

## EFFECTS OF DISTURBING FACTORS (SUCCESSIONS)

In Coachella Valley, when a typical desert-sage area is destroyed by fire or cultivation, *Isocoma veneta acradenia* (Greene) H. M. Hall takes a prominent place in the successions preceded by annuals and biennials and followed by desert-sage. This is well illustrated when the vegetation along the roads (Pl. 6, A) is destroyed for use in "brushing" the roads. The strips where the vegetation has been destroyed are then dominated by *Isocoma*, while the rest remains as virgin desert-sage.

In places having more favorable moisture conditions, that is, land formerly cultivated, the seepweed, arrowweed, or saltbush may act as a weed in an early stage of the successions until the moisture is somewhat exhausted, which may then be followed by *Isocoma*, and, finally, by desert sage.

## VARIATIONS FROM THE TYPICAL ASSOCIATIONS

The desert-sage mixes with the creosote bush association above it, and with the seepweed association below. The former has been discussed as a variation of the creosote bush association; the latter will be discussed under the seepweed association.

## MESQUITE THICKET

## TOPOGRAPHICAL RELATIONS

The mesquite thicket has a wider range than the creosote bush, extending as far west as the creosote bush and much farther east into Texas. The mesquite thickets occupy the lowest land in the valleys. The largest and densest areas occur along the river bottoms where the land is subirrigated, but these give way to desert-sage as the land rises gradually toward the mountains.

## BOTANICAL COMPOSITION

A typical mesquite thicket contains no other trees or shrubs than the mesquite (*Prosopis glandulosa* Torr.). In a large part of the area where the mesquite dominates, however, there are several other shrubs mixed with it. Here *Atriplex polycarpa* (Torr.) S. Wats., *Dondia intermedia* (S. Wats.) Heller, *Atriplex linearis* S. Wats., and *Lycium parviflorum* A. Gray are found on low hummocks between the mesquite trees. These hummocks are accumulations of light soil, usually sand, blown about by the wind and lodged around the bushes.

## APPEARANCE

The appearance of a mesquite thicket depends on the abundance of the trees. Where conditions are favorable the mesquite trees form impenetrable thickets.

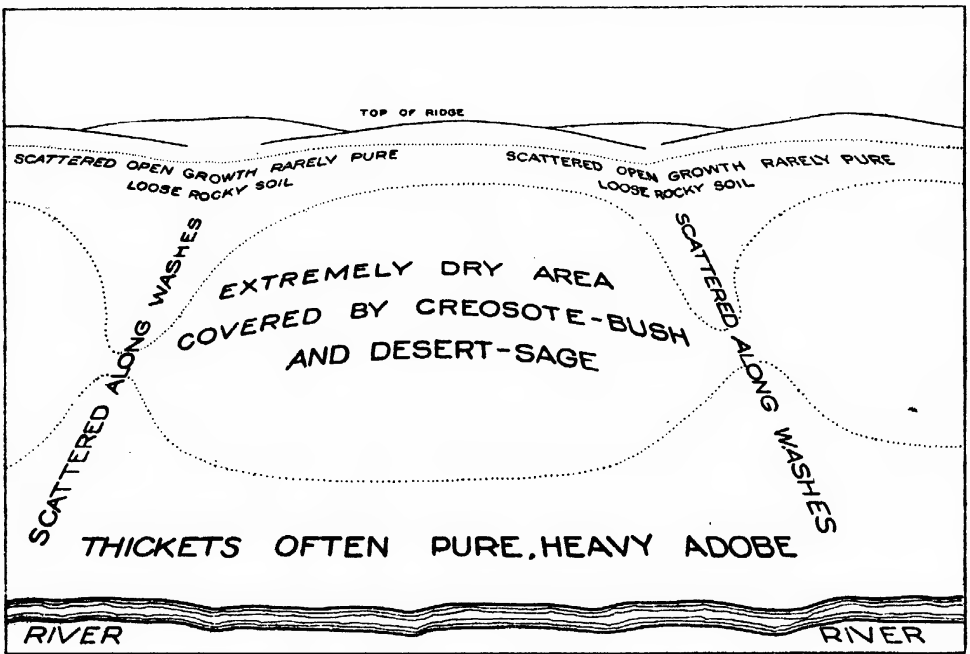


FIG. 13.—A sketch illustrating the distribution of mesquite in desert valleys. This distribution is dependent on soil moisture, either on a high water table, where the soil is heavy or, where the soil is loose and rocky as at the base of slopes and along water courses, on the deep penetration of flood waters following rains.

But in the large areas where the mesquite mixes with the desert-sage, there are wide open spaces covered with hummocks of desert-sage and sometimes seepweed.

## PHYSICAL CONDITIONS INDICATED

## Soil Moisture

Like the seepweed, the mesquite grows in two very distinct zones, with regard to soil moisture: (a) Areas in the lowlands where the water table is high, but not high enough for such shrubs as saltbush, seepweed or arrowweed. Here the growth is best, forming dense, often impenetrable, thickets. The soil is heavy, a clay loam, and usually saline, but not excessively so; (b) areas along washes and on hillsides where it is mixed with other plants forming the "hills mixed" association just above the creosote bush association or the areas in the sandhills where it is mixed with chamiso (Fig. 13). The soil of the areas in the second group is just the opposite of the bottom lands, being either almost pure sand or in the "hills mixed" areas a stony, loose loam, well drained and non-

saline. The penetration of water in the rainy season is deep but the water table is beyond the reach of the root system. The growth here is scattered and open, the individual trees being smaller, scarcely forming a trunk and resembling large shrubs.

The texture of soil indicated by the mesquite thickets is like that of the desert-sage land. Sample 14, Table XXVII, shows the first foot considerably lighter, because it was taken on a hummock, otherwise it would show heavier soil. The main difference in the two types of land is in the soil moisture. In the mesquite land the water table influences the soil moisture. The third and fourth feet were not found dry as late as October. In spring there is an abundance of water in all 4 feet.

#### Salinity.

The salt content is appreciable in all 4 feet and is especially high in the third and fourth feet (sample 14, Table XXVII).

#### Summary of Physical Conditions Indicated

The mesquite thickets indicate land with (1) a soil texture approximately that of desert-sage land; (2) water table high but never actually coming to the surface of the soil; (3) an abundance of available water in all 4 feet in spring, and some water in the third and fourth feet even late in fall; (4) an appreciable quantity of salt in all 4 feet, the quantity increasing in the third and fourth feet.

#### ADAPTATIONS TO PHYSICAL CONDITIONS

The mesquite has a very wide range of adaptability as to the soil conditions under which it grows, from a nonsaline type that is practically pure dune sand to the saline heavy adobe lands at the bottoms of the valley. It is on the latter type that the pure dense mesquite thickets occur, often so dense as to be almost impenetrable.

The mesquite puts out a new set of leaves in the early spring and carries a dense foliage throughout the hot months. Most of these leaves are lost in the winter. Mesquite blooms in the spring, and in late summer or early fall bears ripe fruit pods which were used to a large extent as food by the Indians. This tree thrives best on a heavy soil that is subirrigated, and rarely forms a good sized tree on lands where its roots can not reach underground water.

Mesquite thicket land covers considerable areas in the Gila Valley. This land is subject to flooding by the water from lands above, but could probably be used for growing crops after leaching out some of the salts without providing artificial drainage.

#### NARROWLEAF SALTBUSH ASSOCIATION

##### TOPOGRAPHICAL RELATIONS

Narrowleaf saltbush (*Atriplex linearis* S. Wats.), has a much more limited range than desert-sage. It is not found in Coachella Valley. In the Gila Valley it occupies the low flat depressions occurring either in the creosote bush or in the desert-sage areas. The center of these flat areas is either a "bare flat" ("slick land") or a seepweed area. Surrounding this is the narrowleaf saltbush area and outside of this area again is a scrubby growth of creosote bush or desert-sage. At the lines of contact between these types are considerable areas of narrowleaf saltbush mixed with seepweed or desert-sage, and, more rarely, with creosote bush.

##### BOTANICAL COMPOSITION

Typical areas of narrowleaf saltbush (*Atriplex linearis* S. Wats.) contain no other shrubs. Usually *Atriplex polycarpa* (Torr.) S. Wats. *Prosopis glandulosa*

Torr., *Dondia intermedia* (S. Wats.) Heller and *Lycium parviflorum* A. Gray are scattered throughout the area. In places the annual *Atriplex fasciculata* S. Wats. is abundant after the summer rains.

#### APPEARANCE

At a distance narrowleaf saltbush areas are hard to separate from those of desert-sage. At closer range the narrowleaf saltbush appears much smaller and lighter colored, but it has larger leaves than the desert-sage and more of them are retained so that less of the dark stems show.

#### PHYSICAL CONDITIONS INDICATED

##### Soil Moisture

Narrowleaf saltbush indicates a hard compacted red soil at from 4 to 18 inches below the surface. The soil above is loose and lighter. In fall all 4 feet were dry with no moisture available. There are indications that water collects in these areas after the rains so that at this time there is a good supply of available moisture, at least in the upper lighter soil.

##### Salinity

The salt content of the lighter layer above the red compacted soil is not very high (Table XV), but that of the compacted subsoil is considerably higher. Where narrowleaf saltbush mixes with creosote bush the salt content is much lower (Table XVI).

#### Summary of Physical Conditions Indicated

Typical areas of narrowleaf saltbush indicate: (1) A heavy compacted subsoil with a lighter layer of soil 4 to 18 inches in depth; (2) no available moisture in the fall in any of the 4 feet of soil but probably a good supply in the upper, light layer of soil after the rainy season; (3) a small quantity of salts in the light layer of soil but ranging over  $\frac{1}{2}$  per cent in the compact subsoil. Narrowleaf saltbush indicates a much shallower soil than desert-sage.

TABLE XV.—*Soil conditions in typical areas of narrowleaf saltbush, Casa Grande, 1915*<sup>a</sup>

Item	Depth of soil	Date of collection		Average
		Oct. 11 sample No. 3 <sup>b</sup>	Oct. 12 sample No. 15	
	<i>Feet</i>			
Moisture equivalent.....	1	21.9	21.4	21.7
	2	21.4	25.6	23.5
	3	18.3	22.5	20.4
	4	20.4	31.3	25.8
Wilting coefficient.....	1	11.9	11.6	11.8
	2	11.6	13.9	12.8
	3	9.9	12.2	11.1
	4	11.1	17.0	14.1
Moisture content above or below wilting coefficient.....	1	d	<sup>c</sup> d	-----
	2	d	d	-----
	3	d	d	-----
	4	d	d	-----
Salt content.....	1	.15	.17	.16
	2	.34	.24	.29
	3	.22	.98	.60
	4	.25	.91	.58

<sup>a</sup> All data in percentages of the dry weight of the soil.

<sup>b</sup> Sample No. 3 was taken in a narrowleaf saltbush flat surrounded by creosote bush. See Table XVI for sample taken where narrowleaf saltbush and creosote bush mix at the border line of this same flat. Sample No. 15 was taken in a narrowleaf saltbush flat surrounded by desert-sage.

<sup>c</sup> d=dry soil.



TABLE XVI.—Soil conditions in an area of narrowleaf saltbush mixed with a poor growth of creosote bush, Casa Grande, Ariz., 1915 <sup>a</sup>, <sup>b</sup>

Item	Depth of soil	Date of collection Oct. 11, sample No. 4
Moisture equivalent.....	<i>Feet</i>	
	1	31.4
	2	22.2
	3	35.7
	4	32.2
Wilting coefficient.....	1	17.1
	2	17.5
	3	19.4
	4	17.5
Moisture content above or below wilting coefficient.	1	<sup>c</sup> d
	2	d
	3	d
	4	d
Salt content.....	1	.11
	2	.20
	3	.24
	4	.36

<sup>a</sup> All data in percentages of the dry weight of the soil.  
<sup>b</sup> The creosote bush measured from 1 to 2½ feet high.  
<sup>c</sup> d=dry soil.

ADAPTATIONS TO PHYSICAL CONDITIONS

Narrowleaf saltbush has a very shallow root system limiting itself to the upper layer of loose soil. More leaves are retained than in the case of the desert-sage, and the fruits here are not produced in as great quantities.

SALTGRASS ASSOCIATION

TOPOGRAPHICAL RELATIONS

Large saltgrass areas are not as common in the Southwestern desert region as they are in the Great Basin.

In the Gila Valley the saltgrass areas are insignificant, occurring only as scattered small meadows along the river. In Coachella Valley the pure areas of saltgrass, that is, the meadows, are small, rarely several acres in extent, and are scattered throughout the flat and poorly drained land, the area covered by the pickleweed, the saltbush, and the arrowweed. Saltgrass also occurs in all parts of the valley around springs, flowing wells, and reservoirs.

BOTANICAL COMPOSITION

The saltgrass meadows are usually a sod of pure *Distichlis spicata* L. Greene, but sometimes there are scattered plants of *Cressa truxillensis* H. B. K. *Allenrolfea occidentalis* (S. Wats.) Kuntze, *Dondia torreyana* (S. Wats.) Standl., *Dondia intermedia* (S. Wats.) Heller, *Pluchea sericea* (Nutt.) Coville, or *Atriplex lentiformis* (Torr.) S. Wats. may be mixed in at the outer edge, according to which of the plant groups border the meadow.

APPEARANCE

The meadows show a very uniform growth of saltgrass, practically excluding all other plants (Pl. 8, A.)

In late winter and early spring (January, February, and March) the meadows have a "cured grass" color, the saltgrass having made but little growth. Later in the season, however, and until late fall the color is fresh green.

PHYSICAL CONDITIONS INDICATED

Soil Moisture

Saltgrass indicates a heavy soil where the water table comes up to or very near the surface. There is an abundance of available water all the year; even the first foot rarely becomes dry (Tables XVII and XVIII).

Salinity

The salt content of saltgrass land is higher than that of any other type of land, with the exception of pickleweed land (Table XXXIV). The average salt content of the 4 feet of soil is over 1 per cent (Tables XVII and XVIII.) Considerable quantities of black alkali often occur in the third and fourth feet, as, for instance, in Table I, sample 8, which represents the fourth foot in a saltgrass meadow, over 26 per cent of the total salt content were bicarbonates and 6 per cent carbonates.

Summary of Physical Conditions Indicated

Saltgrass land is very saline and has a high water table. In order to use this land for growing farm crops profitably, it is necessary to drain the land and leach out some of the salts. Saltgrass, however, is eaten by stock, so that this land in its natural state affords pasturage. In some places it is even cut for hay.

ADAPTATIONS TO PHYSICAL CONDITIONS

The range of tolerance of saltgrass is very great. On salt flats it is often found pushing into the most extremely saline soils. Where flood water is carried over an area it develops a relatively luxuriant growth, and it will do well in nonsaline soils. This grass spreads by root stalks, and these are pushed gradually out into the salt flats. It also has the ability to remain dormant for a long period and grow again when rain or flood waters leach to some extent the saline surface soil and replenish the soil moisture supply.

TABLE XVII.—Soil conditions in September and October in typical areas of salt-grass, Indio, Calif., 1914 <sup>a</sup>

Item	Depth of soil	Date of collection			Average
		Sept. 14, sample No. 12	Oct. 13, sample No. 77	Oct. 14, sample No. 86	
Salt content.....	<i>Feet</i>				
	1	>2.50	>2.50	0.39	1.80
	2	.73	1.56	1.40	1.23
	3	.48	1.66	.68	.94
Water content <sup>a</sup> .....	4	.56	1.32	.19	.69
	1	<sup>b</sup> m	<sup>d</sup>	w	-----
	2	m	{ 2/3 d 1/3 m }	w	-----
	3	m	m	w	-----
Soil type <sup>a</sup> .....	4	w	m	w	-----
	1	<sup>c</sup> cl	sl	fsl	-----
	2	cl	sl	fsl	-----
	3	c	l	sl	-----
	4	c	sl	sl	-----

<sup>a</sup> No soil moisture or moisture equivalent determinations were made on the soil samples of 1914. The data given are merely the field notes on the samples. Salt content data are stated in percentages of the dry weight of the soil.  
<sup>b</sup> m=moist soil; d=dry soil; w=wet soil.  
<sup>c</sup> s=sand or sandy; l=loam; fsl=fine sandy loam; c=clay.

TABLE XVIII.—*Comparison of spring and fall soil conditions at the same stations in typical areas of saltgrass, Indio, Calif., 1915* <sup>a</sup>

Item	Depth of soil	Date of collection		Average	Date of collection		Average
		Mar. 5, sample No. 21	Mar. 5, sample No. 22		Sept. 22, sample No. 21	Sept. 22, sample No. 22	
Moisture equivalent.....	<i>Feet</i>						
	1	27.1	27.1	27.1	23.5	35.8	29.7
	2	14.9	27.6	21.3	13.8	26.7	20.3
	3	36.7	34.2	35.5	29.0	31.2	30.1
Wilting coefficient.....	4	16.4	28.6	22.5	13.7	28.2	21.0
	1	14.7	14.7	14.7	12.8	19.5	16.2
	2	8.1	15.0	11.6	7.5	14.5	11.0
	3	20.0	18.6	19.3	15.8	17.0	16.4
Moisture content above or below wilting coefficient.	4	8.9	15.6	12.3	7.4	15.3	11.4
	1	+18.3	+14.2	+16.3	+10.1	+0.7	+5.4
	2	+22.2	+12.6	+17.4	+13.7	+5.8	+9.8
	3	+16.8	+17.3	+17.1	+18.0	+10.5	+14.3
Salt content.....	4	+24.6	+14.1	+19.4	+19.4	+16.0	+17.7
	1	>2.50	2.40	2.45	>2.50	1.66	2.08
	2	2.24	.30	1.27	1.90	.74	1.32
	3	.41	.30	.36	1.16	.89	1.03
	4	.26	.13	.20	.47	.58	.53

<sup>a</sup> All data in percentages of the dry weight of the soil.

WASHINGTON PALMS

TOPOGRAPHICAL RELATIONS

The Washington palm is peculiar to Coachella Valley. The palms occur as scattered groups around springs or streams. The most of these are in the part of the valley above Indio, although in groups of two or three they extend down beyond Mecca. The most striking groups are those north of Indio where the palms make extensive groves just next to the barren clay hills (Pl. 9). Large groups are also found above Palm Springs where the palms follow the mountain streams for several miles.

BOTANICAL COMPOSITION

Where the palm trees (*Washingtonia filifera* Wendl.) grow around springs in the heavier soils there is usually a sod of *Distichlis spicata* (L.) Greene. In the group north of Indio *Juncus robustus* S. Wats. and *Imperata hookerii* Rupr. are associated with the *Distichlis*. On the lower places the *Distichlis* is mixed with *Juncus cooperi* Engelm. and *Scirpus olneyi* A. Gray. Along the streams in the hills the soil is rockier and the vegetation growing with the palms is characteristic of the washes, previously discussed under the Yucca and cactus association.

APPEARANCE

Because of the height and the contrast with the low, shrubby vegetation surrounding them, the palm groups make a striking appearance and can be seen for long distances. This is not true of the one or two trees often found at the springs in the flats, where scattered mesquite trees tend to hide the palms. The trees are often 50 feet or more in height, with a trunk about 2 feet in diameter. At the end of the growing season the leaves merely droop and dry but do not fall off, so that the upper part of the trunk is entirely hidden by the mass of old leaves (Pl. 9).

PHYSICAL CONDITIONS INDICATED

Soil Moisture

These palm groups always indicate a good supply of moisture either from springs or mountain streams available all the year.

### Salinity

A single test of the upper 15 inches of soil taken in the group of palms north of Indio showed only a small amount of salt, 0.16 per cent. The surface of the soil where this group occurs is crusted with white salt, mostly sulphates (Table I, sample 4).

#### SUMMARY OF PHYSICAL CONDITIONS, AND ADAPTATIONS

The palm trees indicate a good supply of water available all the year. The soil, although saline, is not excessively so, due probably to the constant supply of comparatively fresh water from the subsoil.

Since water is available throughout the summer and since high temperatures are necessary for the palm's growth, most of its growth is made during the summer.

#### PICKLEWEED ASSOCIATION

##### TOPOGRAPHICAL RELATIONS

The pickleweed areas, like the saltgrass areas, are not as common or extensive in the Southwestern desert region as in the Great Basin. Pickleweed covers the central and lower portions of Coachella Valley (fig. 2). The area covered is a more or less continuous strip, very broad at the edge of the Salton Sea, where it is coming in as an early stage of a succession on the saline soil as the water recedes, and narrowing toward the upper part of the valley into a narrow strip along the bottom of a dry creek. In the Gila Valley and throughout the rest of the Southwestern desert region there is but little pickleweed, and where it does occur it is mixed with other plants in small areas usually along the rivers.

##### BOTANICAL COMPOSITION

While most of this area in Coachella Valley is pure pickleweed (*Allenrolfea occidentalis* (S. Wats.) Kuntze) it is often associated with scattered plants of saltgrass (*Distichlis spicata* (L.) Greene), arrowweed (*Pluchea sericea* (Nutt.) Coville), *Atriplex lentiformis* (Torr.) S. Wats., *Atriplex canescens* (Pursh) Nutt. and *Dondia torreyana* (S. Wats.) Standl. No annuals are found here because of the high salt content of the surface of the soil.

##### APPEARANCE

The areas in Coachella Valley consist of large flat tracts bearing scattered plants of pickleweed. The spaces between the plants are white with a heavy coating of salts (Pl. 8, B). A typical area shows dark bushy brownish-green plants from 1 to 2 feet high. Large tracts of this kind are monotonous in appearance. In the early spring the plants are brown, the parts above ground dying in winter.

##### PHYSICAL CONDITIONS INDICATED

##### Soil Moisture

The soil is more permeable than that of the desert-sage land. The texture in the third and fourth feet is considerably lighter than that of the desert-sage land (Table XIX). The soil moisture (Tables XIX and XX) is greatly increased by the high water table. In spring the water comes almost to the surface and the land is very boggy, more so than any other type of land in the valley. While the saltgrass land is very wet, the sod formed tends to make it firmer on the surface. In the fall the surface foot of soil is no longer boggy, but there is a good supply of available moisture in all 4 feet of soil.

**TABLE XIX.**—*Comparison of spring and fall soil conditions at the same stations in typical areas in the pickleweed association, Indio, Calif., 1915<sup>a</sup>*

Item	Depth of soil	Date of collection			Average	Date of collection			Average
		Mar. 4, sample No. 26	Mar. 5, sample No. 27	Mar. 5, sample No. 28		Sept. 22, sample No. 26	Sept. 22, sample No. 27	Sept. 23, sample No. 28	
Moisture equivalent.....	<i>Feet</i>								
	1	21.1	21.7	26.4	23.1	21.5	18.9	21.5	20.6
	2	16.0	24.1	29.1	23.1	20.0	19.9	29.9	23.2
	3	21.2	11.7	21.6	18.2	23.2	11.0	20.1	18.1
Wilting coefficient.....	4	20.0	13.1	17.8	17.0	7.1	9.1	15.0	10.4
	1	11.5	11.8	14.4	12.6	11.7	10.3	11.7	11.2
	2	8.7	13.1	15.8	12.5	10.9	10.8	16.3	12.7
	3	11.5	6.4	11.8	9.9	12.6	6.0	10.9	9.8
Moisture content above or below wilting coefficient.	4	10.9	7.1	9.7	9.2	3.9	5.0	8.1	5.6
	1	+16.1	+15.3	+21.0	+17.5	+1.8	+8.2	+7.3	+5.8
	1	+12.3	+16.0	+20.0	+16.1	+5.2	+10.8	+11.2	+9.1
	3	+12.6	+24.0	+18.5	+18.4	+7.1	+15.3	+17.3	+13.2
Salt content.....	4	+15.7	+21.7	+25.1	+20.8	+6.2	+12.5	+16.7	+12.0
	1	>2.50	>2.50	>2.50	>2.50	>2.50	>2.50	>2.50	>2.50
	2	>2.50	2.50	.59	1.86	2.20	1.73	.20	1.38
	3	>2.50	.65	.36	1.17	1.45	1.10	.20	.92
	4	.68	.36	.40	.48	.24	.91	.18	.44

<sup>a</sup> All data in percentages of the dry weight of the soil.**TABLE XX.**—*Soil conditions in September and October in typical areas of the pickleweed association, Indio, Calif., 1914<sup>a</sup>*

Item	Depth of soil	Date of collection							Average
		Sept. 14, sample No. 9	Sept. 22, sample No. 32	Oct. 2, sample No. 58	Oct. 14, sample No. 85	Oct. 15, sample No. 89	Oct. 15, sample No. 94	Oct. 31, sample No. 103	
Salt content.....	<i>Feet</i>								
	1	1.90	2.10	>2.50	.36	>2.50	2.10	1.01	1.78
	2	.40	1.52	1.96	1.73	1.04	>2.50	1.10	1.46
	3	.21	2.30	.70	1.50	.76	1.96	1.01	1.21
Water content.....	4	.18	1.56	.29	.68	.16	1.56	1.04	.78
	1	m <sup>b</sup>	d	w	w	w	d	w	-----
	2	w	m	w	w	w	sm	w	-----
	3	w	m	w	w	w	m	w	-----
Soil type.....	4	w	m	w	w	w	m	w	-----
	1	l <sup>c</sup>	fsl	fsl	sl	sl	sl	c	-----
	2	l	fsl	fsl	sl	sl	sl	c	-----
	3	sl	fsl	fsl	fsl	fsl	fsl	c	-----
	4	l	fsl	fsl	fsl	fs	fsl	c	-----

<sup>a</sup> No soil moisture or moisture equivalent determinations were made on the soil samples of 1914. The data given are merely the field notes on the samples. Salt content data are stated in percentages of the dry weight of the soil.<sup>b</sup> m=moist soil; d=dry soil; sm=slightly moist soil; w=wet soil.<sup>c</sup> l=loam; s=sand or sandy; f=fine; c=clay.

### Salinity

Pickleweed indicates land that has an excessive amount of salt in the first and second feet, and often the third (Tables XIX and XX). The fourth foot is least saline, averaging approximately one-half of 1 per cent (Table XXXIV). The average salt content for the 4 feet of soil is about 1½ per cent. In places there is a uniform white layer of salt on the surface that resembles a covering of snow.

## Summary of Physical Conditions

Pickleweed indicates land with a lighter texture than that of the desert-sage, with a high-water table that comes to the surface in early spring and furnishes an abundant supply throughout the year, and an excessive salt content rarely less than 1 per cent in the first foot and averaging  $1\frac{1}{2}$  per cent for all 4 feet of soil.

## ADAPTATIONS TO PHYSICAL CONDITIONS

Pickleweed is limited to soils where the water table reaches the surface of the soil for at least a part of the year. The soil surface is typically encrusted with salt. It has a relatively deep root system and is able to thrive in a water-saturated, strongly saline soil (Pl. 10, A). It can endure more water and more salt than seepweed.

## MESQUITE AND CHAMISO COMMUNITY

## TOPOGRAPHICAL RELATIONS

This type of vegetation covers the sand hills of the region. In Coachella Valley (fig. 2) the greater part of this area lies in the upper part of the valley, below the creosote bush and breaking in on what would otherwise be occupied by desert-sage. At Myoma the area extends practically across the valley. The hills themselves vary a great deal in size and form, from the low mounds representing the old dunes covered with vegetation to the barest hills formed by the newly drifted sand. They are formed by the light soil picked up by the winds blowing down San Gorgonio Pass and deposited some distance farther down the valley.

In the Gila Valley (fig. 3) a modification of this association is of minor importance. It covers low sandy knolls and ridges scattered in the valley just southwest of Chandler, and is in the midst of the desert-sage association.

## BOTANICAL COMPOSITION

There are several important woody plants in this area as well as a large number of annuals. The chamiso (*Atriplex canescens* (Pursh) Nutt.) is the most important woody species in this type of vegetation where the slope is not so steep. The mesquite (*Prosopis glandulosa* Torr.) covers the tops of the sandhills and, sometimes, all of the lower and older ones. On some of the oldest hills creosote bush is the most important shrub. *Isocoma veneta acradenia* (Greene) H. M. Hall is important in places and *Parosela emoryi* (A. Gray) Heller quite frequent. The areas of depression between the hills where silt has been washed in is occupied by desert-sage (*Atriplex polycarpa* (Torr.) S. Wats.)

The number of species of annuals is large, comparing favorably with the creosote bush land. They do not cover the ground as completely as on creosote bush land, but on the contrary are often widely spaced, no single species predominating. The plants found in this area are given in the following list:

## PERENNIAL SPECIES OF THE MESQUITE AND CHAMISO

## Common or Frequent

<i>Prosopis glandulosa</i> Torr.	<i>Psathyrotes ramosissima</i> (Torr.) A. Gray.
<i>Atriplex canescens</i> (Pursh) Nutt.	<i>Atriplex polycarpa</i> (Torr.) S. Wats.
<i>Covillea glutinosa</i> (Engelm.) Rydb.	<i>Parosela emoryi</i> (A. Gray) Heller.
<i>Isocoma veneta acradenia</i> (Greene) H. M. Hall.	<i>Geraea eriocephala</i> (A. Gray) Blake.
<i>Abronia villosa</i> S. Wats.	

## Less Frequent or Rare

<i>Atriplex hymenelytra</i> (Torr.) S. Wats.	<i>Parosela arborescens</i> (Torr.) Heller.
<i>Chilopsis linearis</i> (Cav.) Sweet.	<i>Petalonyx thurberi</i> A. Gray.
<i>Isomeris arborea</i> Nutt.	<i>Lepidospartum squamatum</i> A. Gray.

## ANNUAL AND BIENNIAL SPECIES OF THE MESQUITE AND CHAMISO

## Common or Frequent

<i>Anogra trichocalyx</i> (Nutt.) Small.	<i>Eremocarya micrantha</i> (Torr.) Greene.
<i>Chylisma scapoidea</i> (Nutt.) Small.	<i>Palafoxia linearis</i> Lag.
<i>Dithyrea californica</i> Harv.	<i>Achyronychia cooperi</i> Torr. & Gray.
<i>Sphaerostigma veitchianum</i> (Hook.) Small.	<i>Eriogonum thomasi</i> Torr.
<i>Cryptanthus</i> sp.	<i>Eremalche exilis</i> (A. Gray) Greene.

## Less Frequent or Rare

<i>Baileya panciradiata</i> Harv. & Gray.	<i>Sophia pinnata</i> (Walt.) Howell.
<i>Calyptridium monandrum</i> Nutt.	<i>Stillingia annua</i> Muell. Arg.
<i>Croton californicus</i> Muell. Arg.	<i>Thelypodium lasiophyllum</i> (Hook. & Arn.) Greene.
<i>Plantago erecta</i> Morris.	

## APPEARANCE

The chamiso is greener than the desert-sage and is considerably taller. In early spring, just after the new growth has come on, the plants are very green. In the latter part of summer most of the leaves have dropped (Pl. 11, B) but large quantities of fruits cover the smaller branches. In favorable years the fruit is so abundant that the branches bend under its weight.

The mesquite trees growing on the soil occupied by the mesquite and chamiso association have a distinct form. On the drier heavier soils occupied by the desert-sage, or the wetter saline soils occupied by the saltbush and arrowweed, the mesquite grows as a tree with a single large trunk often more than 13 inches in diameter. On the sand hills, however, the mesquite is shrubby, sprawling over large spaces of ground and forming small thickets. On some of the newer dunes these are partly buried under the sand so that only the smaller branches are seen above the surface (Pl. 10, B). The mesquite begins growth only after warm weather sets in, as the leaves do not appear until March. Up to that time they are leafless and reddish-brown, due to the color of the bark of the branches and twigs. From March on, throughout the long hot summer, they retain a heavy foliage.

## PHYSICAL CONDITIONS INDICATED

## Soil Moisture

The soil is practically a pure sand on the hills with a little silt in the depressions. It is very readily permeable, with practically no run-off, and has a very low moisture holding capacity, as is shown in Table XXI. Immediately after the winter rains there is water available to a depth of 4 feet. The surface foot is dried out rapidly by the annuals and by evaporation. By summer there is no available water to a depth of 4 feet. However, in cases where the hills are bare and the water has not been used by the plants there is available water in the third and fourth feet even as late as September (sample 16, Table XXI).

TABLE XXI.—Comparison of spring and fall soil conditions at the same stations in typical areas of mesquite and chamiso, Indio, Calif., 1915 <sup>a</sup>

Item	Depth of soil	Date of collection					Average	Date of collection					Average
		Mar. 3, sample No. 16	Mar. 2, sample No. 17	Mar. 2, sample No. 18	Mar. 2, sample No. 19	Mar. 2, sample No. 20		Sept. 20, sample No. 16	Sept. 21, sample No. 17	Sept. 21, sample No. 18	Sept. 23, sample No. 19	Sept. 23, sample No. 20	
Moisture equivalent	<i>Feet</i>												
	1	2.3	2.0	2.6	2.1	2.7	2.3	2.4	3.1	3.3	2.2	2.2	2.6
	2	2.9	3.0	2.9	2.5	2.3	2.7	2.9	3.3	2.7	2.6	2.7	2.8
	3	2.8	3.4	2.5	2.2	1.9	2.6	3.3	3.9	1.9	2.7	2.4	2.8
Wilting coefficient..	4	2.6	3.1	3.0	2.6	2.6	2.8	3.8	4.5	2.2	2.6	2.3	3.1
	1	1.3	1.1	1.4	1.1	1.5	1.3	1.3	1.7	1.8	1.2	1.2	1.4
	2	1.6	1.6	1.6	1.3	1.3	1.5	1.6	1.8	1.5	1.4	1.5	1.6
	3	1.5	1.9	1.4	1.2	1.0	1.4	1.8	2.1	1.0	1.5	1.3	1.5
Moisture content above or below wilting coefficient..	4	1.4	1.7	1.6	1.4	1.4	1.5	2.1	2.4	1.2	1.4	1.3	1.7
	1	+2.5	+2.2	+3.1	+2.1	+2.1	+2.4	-1.2	-1.5	-1.7	-1.0	-1.1	-1.3
	2	+3.8	+3.8	+3.5	+3.9	+3.9	+3.8	0.0	-1.6	-1.0	-1.2	-1.1	-1.0
	3	+4.1	+5.9	+2.4	+3.0	+4.8	+4.1	+0.9	-1.7	+0.5	-1.2	0.0	-0.3
Salt content-----	4	+0.8	+1.0	+0.1	-0.2	+0.5	+0.4	+0.8	-1.6	+1.1	-0.9	-0.1	-0.1
	1	<.01	<.01	<.01	<.01	<.01	<.01	<.01	<.01	.02	<.01	<.01	.01
	2	<.01	<.01	<.01	<.01	.01	<.01	.01	<.01	<.01	<.01	.02	.01
	3	.02	<.01	<.01	<.01	.06	.02	<.01	.01	<.01	<.01	.02	.01
	4	.02	.03	.02	.02	.02	.02	.02	.02	.12	<.01	.01	.04

<sup>a</sup> All data in this table are stated in percentages of the dry weight of the soil.

Salinity

The salt content of the soils of this area is very low (Table XXI), which is expected in soil that is practically pure sand.

Summary of Physical Conditions

The lands covered by mesquite and chamiso show: (1) Practically a pure sand with very good penetration and low run-off, but low moisture-holding capacity; (2) a good supply of moisture after the winter rains to a depth of 4 feet; (3) a very low salt content.

ADAPTATIONS TO PHYSICAL CONDITIONS

The chamiso begins growth much earlier than the mesquite and because of its shallower root system (Pl. 11, B) makes use of the moisture collected in the upper 4 feet of soil during the rainy season. As soon as the weather becomes hot some of the lower leaves fall off so that by late summer the plants are almost leafless but bear large quantities of fruit.

Since the moisture supply of the mesquite is not so limited, and since it sends its roots to a greater depth, it is therefore much less conservative than the chamiso in regard to water loss in the hot months of the year.

The mesquite and chamiso land in the Coachella Valley is capable of crop production where the sand hills are not too high. Some of the lower and older dunes where water from artesian wells was available have already been leveled and planted to dates, and it is apparent that a large part of this area can be so utilized.



## VARIATIONS FROM THE TYPICAL COMMUNITY

## Chamiso

In the Gila Valley (fig. 3) the chamiso (*Atriplex canescens* (Pursh) Nutt.) covers long low narrow sandy ridges not at all like the high, wind-blown sand hills of the Coachella Valley. Here the half-buried abnormal growth of the mesquite is lacking. Chamiso is often the only shrubby species, though frequently there is found an *Isocoma wrightii* (A. Gray) Rydb. and, more rarely, *Baccharis sarothroides* A. Gray or *Sphaeralcea coulteri* A. Gray. The annuals are abundant (Pl. 11, A). Among them are some showy large-flowered species.

The following list includes all the plants found in this community:

## PERENNIAL SPECIES OF CHAMISO

## Common or Frequent

*Atriplex canescens* (Pursh) Nutt.

*Isocoma wrightii* (A. Gray) Rydb.

*Prosopis glandulosa* Torr.

## Less Frequent or Rare

*Baccharis sarothroides* A. Gray.

*Sphaeralcea coulteri* A. Gray.

## ANNUAL AND BIENNIAL SPECIES

## Common or Frequent

*Thelypodium lasiophyllum* (Hook. & Arn.) Greene.

*Pachylophus* sp.

*Mentzelia albicaulis* Dougl.

*Conanthus demissus* (A. Gray) Heller.

*Dipetalia subulata* (Webb & Berth.)

*Pectocarya penicillata* (Hook. & Arn.) A. DC.

Kuntze.

*Machaeranthera parviflora* A. Gray.

*Baeria chrysostoma* Fisch. & Mey.

*Chylisma scapoidea* (Nutt.) Small.

*Festuca octoflora* Walt.

*Amsinckia menziesii* (Lehm.) Nels. & Macbr.

*Anogra albicaulis* (Pursh) Britton.

*Plantago erecta* Morris.

## Less Frequent or Rare

*Eriogonum thurberi* Torr.

*Conanthus demissus* (A. Gray) Heller.

*Erodium texanum* A. Gray.

*Matricaria matricarioides* (Less.) Porter.

*Draba cuneifolia* Nutt.

*Phacelia* sp.

*Lappula occidentalis* (S. Wats.) Greene.

*Sophia glabra* Woot. & Standl.

*Eremalche exilis* (A. Gray) Greene.

*Spermolepis echinatus* (Nutt.) Heller.

The moisture equivalents are low, especially in the upper 2 feet of soil (Table XXII). The moisture-holding capacity therefore is not high, but the penetration is such that it is not likely that any water runs off during rainfall. Available water was found in the first 4 feet of soil in March. In October, available water was present in a very small quantity in the fourth foot only.

In three out of the four borings in this type of land the salt content was very low in all 4 feet. In one case (sample 13, Table XXII) the readings were much higher, especially below the first foot. There was considerable lime in small lumps scattered throughout the lower depths. From the standpoint of growing crops these quantities of salts would not be of great importance.

Chamiso ("sand bars") in the Gila Valley indicates (1) a light sandy soil; (2) soil moisture available in spring in all 4 feet of soil; and (3) a low salt content.

These areas are not extensive enough to be of great importance agriculturally. However, they can be farmed. In some cases they have been leveled and used for truck crops.

## ARROWWEED AND SALTBUSH COMMUNITY

## TOPOGRAPHICAL RELATIONS

This community is unimportant outside of Coachella Valley. It is confined to "seep" areas or subirrigated strips of land along the rivers. The areas in Coachella Valley occupied by the mixture of arrowweed and saltbush occur almost entirely below a line drawn east and west through Thermal (fig. 2). Above this there are only narrow strips along the dry creeks. The areas are very irregular in form and are disconnected, usually skirting the pickleweed area and coming just within that covered by seepweed.

TABLE XXII.—A comparison of spring and fall soil conditions at the same stations in typical areas of chamiso "sandbars," Chandler, Ariz., 1915<sup>a</sup>

Item	Depth of soil	Date of collection				Average	Date of collection				Average
		Mar. 16, sample No. 2	Mar. 25, sample No. 13	Mar. 25, sample No. 18	Mar. 26, sample No. 20		Oct. 1, sample No. 2	Oct. 1, sample No. 13	Oct. 1, sample No. 18	Oct. 7, sample No. 20	
Moisture equivalent...	<i>Feet</i>										
	1	6.4	6.4	6.4	5.5	6.2	6.4	9.4	7.0	6.0	7.2
	2	7.9	8.6	6.7	6.4	7.4	8.2	9.8	7.8	6.7	8.1
	3	15.7	10.0	6.8	6.7	9.8	15.2	12.5	7.4	7.4	10.5
Wilting coefficient.....	4	14.8	14.0	6.0	6.4	10.3	20.2	13.7	7.6	7.9	12.4
	1	3.5	3.5	3.5	3.0	3.4	3.5	5.1	3.8	3.2	3.9
	2	4.3	4.7	3.6	3.5	4.0	4.5	5.3	4.2	3.6	4.4
	3	8.5	5.4	3.7	3.6	5.3	8.3	6.8	4.0	4.0	5.8
Moisture content above or below wilting coefficient.	4	8.0	7.6	3.3	3.5	5.6	11.0	7.4	4.1	4.3	6.7
	1	+2.0	+1.7	-0.2	+1.4	+1.2	-2.7	-4.2	-2.8	-2.8	-3.1
	2	+3.6	+3.7	+1.8	+3.5	+3.2	-2.0	0.0	-1.6	-1.3	-1.3
	3	+5.3	+5.9	+2.6	+2.0	+4.0	-1.5	+2.3	-1.1	-1.0	-0.3
Salt content.....	4	+5.6	+4.9	+3.2	+3.7	+4.4	-1.1	+2.8	-0.5	-0.8	+0.1
	1	.01	.06	.01	.01	.02	.02	.02	.01	.01	.02
	2	.01	.02	.01	.01	.01	.02	.40	.01	.01	.11
	3	.02	.11	.01	.02	.04	.02	.38	.01	.01	.11
	4	.02	.61	.01	.01	.16	.02	.50	.07	.01	.15

<sup>a</sup> All data in this table are stated in percentages of the dry weight of the soil.

## BOTANICAL COMPOSITION

While the arrowweed (*Pluchea sericea* (Nutt.) Coville) (Pl. 12, B) and the saltbush (*Atriplex lentiformis* (Torr.) S. Wats.) (Pl. 12, A) also occur separately as pure types, the areas of each are very small.

In Coachella Valley the two are nearly always intermingled, often with additional plants of *Isocoma veneta acradenia* (Greene) H. M. Hall, *Dondia torreyana* (S. Wats.) Standl. and *Prosopis glandulosa* Torr. Here, as in the case of the seepweed area, the salt crust on the surface of the soil excludes all annuals. In the Gila Valley, shrubs found mixed with the arrowweed and saltbush are *Dondia intermedia* (S. Wats.) Heller, *Isocoma wrightii* (A. Gray) Rydb. and *Atriplex canescens* (Pursh) Nutt. Here, as in Coachella Valley, no annuals were found.

## APPEARANCE

This type of vegetation shows a very rank, dense, shrubby growth, often 6 feet high or higher. In favorable places the shrubbery is so thick as to be scarcely penetrable; more often, however, there are open spaces of several feet between the plants, which are usually covered with a heavy alkaline crust (Pl. 12, A)

## PHYSICAL CONDITIONS INDICATED

## Soil Moisture

The land occupied by the arrowweed and saltbush type of vegetation in Coachella Valley is characterized by the same soil texture as that of the pickleweed land. The first 2 feet are of considerably finer texture than the third and fourth (Table XXIV). The soil moisture is influenced to a large extent by the high-water table, so that there is an available water supply all the year round below the surface foot (Tables XXIII and XXIV). In spring, the water comes almost to the surface of the soil, although not as high as in the pickleweed land.

Salinity

The average amount of salt in the first foot of this type of land of all the samples in Coachella Valley is more than 1½ per cent (Table XXXIV). The average for the second foot is over ¾ per cent, and for the third and fourth about ½ per cent. The decrease in salinity is probably due to the light texture of the soil underlying the upper 2 feet and to the constant addition of water that is only slightly saline.

Summary of Physical Conditions

Arrowweed and saltbush land in Coachella Valley are characterized by (1) a soil, the upper 2 feet of which is quite comparable to that of the pickleweed land, but underlying which there is a much coarser soil; (2) a high water-table making moisture available, at least below the surface foot, all the year round; and (3) usually a high salt content in the surface foot, considerably less so in the second, and moderate amounts in the third and fourth feet.

ADAPTATIONS TO PHYSICAL CONDITIONS

In Coachella Valley, the arrowweed and saltbush are usually mixed, especially on lands that have been disturbed or have become saturated from flooding or seepage. On flooded lands these plants remain only until the water is exhausted and then give way to the desert-sage or more drought-resistant shrubs.

In seepage or constantly flooded areas, if the supply of moisture remains sufficient, the plants develop to their fullest and form the thickets mentioned. Though the soil requirements of these two plants are alike to a great extent, the arrowweed can endure much drier soil conditions, while the saltbush can withstand soils that are much more saline.

TABLE XXIII.—*Soil conditions in September and October in areas of saltbush, mixed areas of saltbush and arrowweed, and in areas of arrowweed, Indio, Calif., 1914<sup>a</sup>*

Item	Depth of soil	Saltbush			Saltbush and arrowweed			Arrowweed							
		Date of collection		Average	Date of collection		Average	Date of collection						Average	
		Sept. 29, sample No. 50	Oct. 15, sample No. 91		Oct. 14, sample No. 82	Oct. 14, sample No. 83		Sept. 15, sample No. 18	Sept. 6, sample No. 60	Oct. 7, sample No. 65	Oct. 7, sample No. 68	Oct. 7, sample No. 69	Oct. 7, sample No. 70		
Salt content.....	Feet														
	1	>2.50	>2.50	>2.50	2.10	0.28	1.19	0.07	0.23	0.26	>2.50	0.43	0.36	0.64	
	2	.19	.68	.44	.21	1.10	.66	.06	.19	.24	1.20	.22	.24	.33	
	3	.24	.19	.22	.22	1.10	.66	.02	.18	.21	.96	.22	.20	.30	
	4	.11	.12	.12	.11	1.28	.70	.12	.22	.18	.82	.20	.20	.29	
Water content.....															
	1	b w	w	-----	sa	w	-----	nd	d	d	w	d	d	-----	
	2	w	w	-----	sa	w	-----	sm	sm	sm	w	d	sm	-----	
	3	w	w	-----	sa	w	-----	sm	sm	sm	w	sm	m	-----	
	4	w	w	-----	sa	w	-----	sm	sm	m	w	m	m	-----	
Soil type.....															
	1	c sl	fsl	-----	fsl	fsl	-----	fsl	sl	fsl	sl	fsl	fsl	fsl	-----
	2	sl	fsl	-----	fsl	fsl	-----	fsl	sl	fsl	sl	fsl	fsl	fsl	-----
	3	sl	fsl	-----	fsl	fsl	-----	fsl	sl	fsl	sl	fsl	fsl	fs	-----
	4	sl	fsl	-----	sl	sl	-----	fsl	e	fsl	sl	fsl	fs	-----	

<sup>a</sup> No soil moisture or moisture equivalent determinations were made on the 1914 soil samples. The data given are the field notes on the soil samples. Salt content data are stated in percentages of the dry weight of the soil.

<sup>b</sup> d=dry soil; nd=nearly dry soil; m=moist soil; sm=slightly moist soil; w=wet soil; sa=saturated soil.

<sup>c</sup> s=sand or sandy; l=loam; fsl=fine sandy loam; c=clay.

TABLE XXIV.—Comparison of the spring and fall soil conditions at the same stations in typical areas of saltbush and arrowweed, Indio, Calif., 1915<sup>a</sup>

Item	Depth of soil	Date of collection			Average	Date of collection			Average
		Mar. 4, sample No. 31	Mar. 4, sample No. 32	Mar. 5, sample No. 33		Sept. 23, sample No. 31	Sept. 23, sample No. 32	Sept. 23, sample No. 33	
Moisture equivalent.....	<i>Feet</i>								
	1	22.5	32.9	29.5	28.3	24.5	32.4	26.5	27.8
	2	10.0	23.6	25.3	19.6	22.2	19.0	24.0	21.7
	3	4.1	20.0	23.9	16.0	14.0	20.8	24.7	19.8
Wilting coefficient.....	4	4.7	13.2	23.0	13.6	25.2	11.8	19.3	18.8
	1	12.2	17.8	16.0	15.3	13.3	17.6	14.4	15.1
	2	5.4	12.8	13.8	10.7	12.1	10.4	13.1	11.9
	3	2.2	10.9	13.0	8.7	7.6	11.3	13.4	10.8
Moisture content above or below wilting coefficient.	4	2.6	7.2	12.5	7.4	13.7	6.4	10.5	10.2
	1	+9.4	+13.3	+19.7	+14.1	+1.9	-8.6	+6.7	0.0
	2	+8.5	+13.2	+15.8	+12.5	+7.1	-2.2	+7.5	+4.1
	3	+4.7	+13.2	+18.1	+12.0	+5.9	-3.8	+10.2	+4.1
Salt content.....	4	+8.1	+18.9	+22.2	+16.4	+8.4	-1.9	+18.4	+8.3
	1	>2.50	>2.50	>2.50	>2.50	>2.50	1.72	2.18	2.13
	2	1.06	.32	.50	.63	2.50	1.04	.52	1.35
	3	0.32	.16	.35	.28	1.10	.19	.20	.50
	4	0.28	.15	.16	.20	1.18	.16	.14	.49

<sup>a</sup> All data in this table are stated in percentage of the dry weight of the soil.

Both of the plants develop in the summer and have dense foliage during the driest part of the year. In November most of the leaves are dead. The plants remain in this dormant condition until warm weather the next season.

Arrowweed and saltbush land, when cultivated for a time and then abandoned, soon reverts to the same type of vegetation without an intermediate stage. If this land is cleared, irrigated, and planted, bare spots eventually appear in the fields. These are due partly to a rise in the water table and partly to an increase in the salt content of the surface soil. To insure permanent cultivation, it is necessary to supply drainage.

VARIATIONS FROM THE TYPICAL ASSOCIATION

Saltbush

In the Gila Valley the arrowweed and saltbush gives way to either areas where saltbush predominates or areas of pure saltbush, in which case the plants are large, luxuriant, and widely spaced (Pl. 12, A). These areas (fig. 3) are even less extensive than in Coachella Valley. They occupy similar situations—that is, the moister irregular areas along rivers or the abandoned fields where the water table is high. The texture of the soil is more like that of the mesquite thicket, having a higher moisture equivalent than that of any of the other types. As regards soil moisture, this land is wetter than mesquite thicket land since, at least in spring, the ground is wet to the surface and apt to be boggy. Even in October there is an abundance of water, in all 4 feet (Table XXV).

The physical conditions of land characterized by saltbush are (1) a soil that is uniform, at least 4 feet deep, and of fine texture; (2) a high water table; (3) an average salt content of  $\frac{3}{4}$  per cent for the first foot, while the average for all 4 feet ranges from  $\frac{1}{2}$  to  $\frac{3}{4}$  per cent.

SEEPWEED

TOPOGRAPHICAL CONDITIONS

Seepweed is widely distributed and grows well both in the Great Basin area and in the Southwestern desert region. In the Coachella Valley seepweed (*Dondia torreyana* (S. Wats. Standl.) forms a narrow and irregular belt on the more level land between the desert-sage and pickleweed land (fig. 2).

Seepweed grows in two quite distinct zones. One is on dry land, where it covers depressions of flat areas bordering *Atriplex linearis* S. Wats. or desert-sage. The other is on wet land (seepage land) bordering pickleweed or saltbush.

In the Gila Valley seepweed (*Dondia intermedia* (S. Wats.) Heller) occupies low flat areas that are poorly drained and are usually covered with water after a rain. This plant is quite frequent wherever the valley floor is a broad plain with very little slope. The pure areas are small, rarely exceeding 40 acres in extent, but are usually surrounded by larger areas mixed with either narrowleaf saltbush or a scrubby growth of desert-sage. These mixed areas are in turn surrounded by areas of pure narrowleaf saltbush or desert-sage. Seepweed also occurs in places where soil conditions and the associated plants are very different from those mentioned, namely, "seepage areas," where it is found with pickleweed, greasewood, saltgrass, or sporobolus. These are usually strips of land along streams, as the Gila River or the Little Gila, or below irrigated tracts of land.

TABLE XXV.—Comparison of spring and fall soil conditions at the same station in a typical area of saltbush, Chandler, Ariz., 1915 <sup>a</sup>

Item	Depth of soil	Date of collection			Average	Date of collection			Average
		Mar. 26, sample No. 21	Mar. 27, sample No. 24	Mar. 17, sample No. 4		Oct. 5, sample No. 21	Oct. 5, sample No. 24	Oct. 5, sample No. 4	
Moisture equivalent.....	Feet								
	1	19.4	21.2	-----	20.3	20.5	21.4	-----	21.0
	2	18.9	20.9	-----	19.9	19.2	21.6	-----	20.4
	3	18.3	22.5	-----	20.4	19.7	21.1	-----	20.4
Wilting coefficient.....	4	22.9	23.3	-----	23.1	22.2	23.6	-----	22.9
	1	10.6	11.5	-----	11.1	11.1	11.6	-----	11.4
	2	10.3	11.4	-----	10.9	10.4	11.7	-----	11.1
	3	10.0	12.2	-----	11.1	10.7	11.5	-----	11.1
Moisture content above or below wilting coefficient.	4	12.5	12.7	-----	12.6	12.1	12.8	-----	12.5
	1	+14.7	+10.0	-----	+12.4	+7.4	+6.0	-----	+6.7
	2	+21.0	+15.9	-----	+18.5	+11.3	+8.5	-----	+9.9
	3	+25.0	+16.8	-----	+20.9	+13.1	+9.6	-----	+11.2
Salt content.....	4	+21.2	+18.2	-----	+19.7	+10.5	+11.8	-----	+11.2
	1	.45	.75	0.64	.61	.38	.97	1.34	.90
	2	.34	.41	.46	.40	1.48	.37	.47	.84
	3	.34	.38	.41	.38	.90	.32	.47	.56
	4	.33	.37	-----	.35	1.80	.31	.49	.87

<sup>a</sup> All data in this table are stated in percentages of the dry weight of the soil.

BOTANICAL COMPOSITION

Pure areas of seepweed, where no other plants are present are small and uncommon. In the Gila Valley plants of *Atriplex linearis* S. Wats., *Atriplex fasciculata* S. Wats., *Aster parviflorus* A. Gray, or, sometimes, *Plantago erecta* Morris are mixed with the seepweed (*Dondia intermedia* (S. Wats.) Heller.) However, when seep weed grows on the "seepage" lands it is mixed with *Allenrolfea occidentalis* (S. Wats.) Kuntze, *Sarcobatus vermiculatus* (Hook.) Torr., *Sporobolus airoides* Torr., *Distichlis spicata* (L.) Greene, *Atriplex lentiformis* (Torr.), S. Wats. and *Pluchea sericea* (Nutt.) Coville.

In Coachella Valley the seepweed (*Dondia torreyana* (S. Wats.) Standl.), outside of the small pure areas, mixes, on the one hand, with plants on wet saline land, as *Distichlis spicata* (L.) Greene, *Allenrolfea occidentalis* (S. Wats.) Kuntze, *Atriplex lentiformis* (Torr.) S. Wats. and *Fluchea sericea* (Nutt.) Coville, and, on the other hand, with plants on dry desert soil as *Atriplex polycarpa* (Torr.) S. Wats. and *Atriplex canescens* (Pursh) Nutt. In fact creosote bush (*Covillea glutinosa* (Engelm.) Rydb.) and the plant groups above the creosote bush area are the only ones with which *Dondia* does not mix. Where it is near Covillea, *Dondia* occupies depressions while *Covillea* grows on hummocks of lighter soil.

#### APPEARANCE

In the wet ("seep") areas, seepweed grows in dense tangled clumps with open spaces between the clumps, the soil being heavily crusted with salt (Pl. 14, A). In summer the plants are very dark green, but in late fall they are tinged with red or purple. Still later most of the leaves are dropped. The new growth comes on late in spring after the creosote bush has made most of its growth. This is probably due to the fact that there is much more moisture for the seepweed to draw on later in the season and also that, due to its location, the soil does not become warm as soon as in the case of the creosote bush land. Wherever seepweed areas are found on dry land the growth is usually not over 16 or 18 inches high, scant but quite uniform. In spring the plants are green but not as dark green as those on the wet areas. The plants on the dry land become tinged with red or purple earlier than those on the wet lands.

#### PHYSICAL CONDITIONS INDICATED

##### Soil Moisture

The texture of the soil indicated by the seepweed ranges from a fine sandy loam to a clay loam, usually somewhat heavier than that indicated by the desert-sage. The amount of available water is considerably greater than on desert-sage soil in the spring. In the fall the soil moisture of desert-sage land is considerably below the wilting coefficient, but the soil moisture of the seepweed land is somewhat above the wilting coefficient, at least in the third and fourth feet (Tables XXVI and XXVII). Where the seepweed grows on seep land there is usually abundant soil moisture all the year.

##### Salinity

Seepweed is a good indicator of saline land. The only exception to this is that seepweed may, as a weed, temporarily cover land once cultivated and then abandoned (Pl. 13, B), in which case it is not always an indicator of salinity (sample No. 44, Table XXVIII). Even when mixed with other plants it rarely fails as an indicator of high salt content of the soil (Table XXIX). In Tables XXVI, samples 11 and 12, and XXVII is shown the salt content on dry land. The first foot has a considerable salt content, at least in fall, while the last 3 feet are high in salt content. The salt content indicated by seepweed on "seep" land (Pl. 13, A) is shown in Table XXVIII, all 4 feet being high in salt content.

##### Summary of Physical Conditions

A rank tangled growth of seepweed indicates (1) a high water table; (2) a high salt content, the average of the 4 feet exceeding 1 per cent, while the first foot may contain over  $2\frac{1}{2}$  per cent (sample 13, Table XXVI).

A scant, low, uniform growth indicates (1) available water in all 4 feet in spring and usually some available water in the third and fourth feet in fall; (2) a high salt content, the average of the 4 feet ranging from  $\frac{1}{2}$  per cent to 1 per cent.

TABLE XXVI.—*Comparison of spring and fall soil conditions at the same stations in typical areas of seepweed, Indio, Calif., 1915<sup>a</sup>*

Item	Depth of soil	Date of collection			Average	Date of collection			Average
		Mar. 4, sample No. 11 <sup>b</sup>	Mar. 4, sample No. 12 <sup>b</sup>	Mar. 5, sample No. 13 <sup>c</sup>		Sept. 24, sample No. 11 <sup>c</sup>	Sept. 24, sample No. 12 <sup>b</sup>	Sept. 22, sample No. 13 <sup>c</sup>	
Moisture equivalent.....	<i>Feet</i>								
	1	11.6	25.8	33.0	23.5	18.6	27.7	35.0	27.1
	2	21.5	29.4	29.8	26.9	21.9	31.8	30.4	28.0
	3	4.9	25.0	32.2	20.7	11.6	17.7	30.0	19.8
Wilting coefficient.....	4	20.2	21.7	34.1	25.3	20.2	24.3	27.3	23.9
	1	6.3	14.0	17.9	12.7	10.1	15.1	19.0	14.7
	2	11.7	16.6	16.2	14.6	11.9	17.3	16.5	15.2
	3	2.7	13.6	17.5	11.3	6.3	9.6	16.3	10.7
Moisture content above or below wilting coefficient.	4	11.0	11.8	18.5	13.8	11.0	13.2	14.8	13.0
	1	+2.9	+7.2	+6.9	+5.7	-6.6	-13.7	-12.1	-10.8
	2	+7.4	+8.5	+8.7	+8.2	-0.8	-12.1	-4.3	-5.7
	3	+1.6	+1.0	+10.0	+4.2	+0.4	-3.8	+3.6	+0.1
Salt content.....	4	+0.3	-4.0	+10.5	+2.3	-1.3	-7.3	+10.3	+0.6
	1	.32	.16	>2.50	.99	.58	.18	>2.50	1.09
	2	.32	.60	1.16	.69	1.16	.94	1.04	1.05
	3	.66	.52	.32	.50	1.13	.67	1.04	.95
	4	1.58	.52	.26	.78	1.40	.58	.27	.75

<sup>a</sup> All data in this table are stated in percentages of the dry weight of the soil.  
<sup>b</sup> Poor growth.  
<sup>c</sup> Rank growth.

TABLE XXVII.—*A comparison of the spring and fall soil conditions at the same station on seepweed areas<sup>a</sup>*

Item	Depth of soil	Chandler, Ariz., 1915. Date of collection		Casa Grande, Ariz. Date of collection Oct. 11, 1915, sample No. 10 <sup>b</sup>
		Mar. 25, sample No. 14 <sup>b</sup>	Oct. 7, sample No. 14 <sup>b</sup>	
Moisture equivalent.....	<i>Feet</i>			
	1	5.4	5.5	16.0
	2	14.7	16.6	16.1
	3	14.9	20.2	16.0
Wilting coefficient.....	4	18.4	21.3	18.2
	1	2.9	3.0	8.7
	2	8.0	9.0	8.7
	3	8.1	11.0	8.7
Moisture content above or below wilting coefficient.....	4	10.0	11.6	9.9
	1	+1.9	-1.9	d <sup>c</sup>
	2	+5.6	-0.6	sm
	3	+4.0	+2.1	sm
Salt content.....	4	+6.1	+2.1	sm
	1	.02	.33	.25
	2	.14	.94	.50
	3	.54	1.16	1.04
	4	1.56	1.46	.70

<sup>a</sup> All data in this table are stated in percentages of the dry weight of the soil.  
<sup>b</sup> Boring No. 14 was made in an area where seepweed was mixed with narrowleaf saltbush and mesquite. Boring No. 10 was made in a typical area of seepweed.  
<sup>c</sup> d=dry soil; sm=slightly moist soil.

TABLE XXVIII.—*Soil conditions in September and October in typical areas of seepweed, Indio, Calif., 1914* <sup>a</sup>

Item	Depth of soil	Date of collection									Average
		Sept. 14, sample No. 13	Sept. 19, sample No. 26	Sept. 22, sample No. 27	Sept. 22, sample No. 31	Sept. 25, sample No. 44	Sept. 30, sample No. 54	Oct. 13, sample No. 76	Oct. 13, sample No. 78	Oct. 14, sample No. 84	
Salt content-----	<i>Feet.</i>										
	1	>2.50	>2.50	2.18	0.93	0.07	>2.50	2.30	0.76	0.23	1.55
	2	>2.50	2.10	1.13	1.18	.12	.20	1.60	1.04	1.36	1.25
	3	.38	1.22	1.56	1.36	.16	.11	.80	1.66	.64	.89
Water content-----	4	.34	1.06	1.32	1.24	.20	.06	1.04	1.18	.52	.77
	1	m <sup>b</sup>	m	d	d	d	w	d	d	d	-----
	2	m	m	m	m	d	w	d	sm	m	-----
	3	m	m	m	m	sm	w	sm	m	m	-----
Soil type-----	4	m	m	m	m	sm	w	sm	w	m	-----
	1	f <sup>c</sup>	fsl	sl	s	fsl	fsl	fsl	sl	fsl	-----
	2	fi	fsl	sl	sl	fsl	sl	fsl	sl	fsl	-----
	3	l	fsl	sl	sl	fsl	sl	fsl	sl	sl	-----
	4	cl	fsl	sl	fsl	fsl	s	fsl	sl	s	-----

<sup>a</sup> No soil moisture or moisture equivalent determinations were made on the soil samples taken in 1914. The data given are the field notes on samples. Salt content data are stated in percentages of the dry weight of the soil.  
<sup>b</sup> m=moist soil; sm=slightly moist soil; d=dry soil; w=wet soil.  
<sup>c</sup> s=sand or sandy; l=loam; fsl=fine sandy loam; c=clay.

Seepweed land is excessively saline. In addition to this, one type of seepweed land also has a high water table. Both of these conditions must be corrected before this type of land can be profitably used for crop production. This land requires drainage first of all, for an attempt to leach out the salts by irrigating would make the land too boggy to be tillable.

ADAPTATIONS TO PHYSICAL CONDITIONS

Seepweed thrives best on land where available moisture is present all the year, at least in the third and fourth feet. On land at one time cultivated or on "seep" land the seepweed grows in tangled clumps, but on virgin desert soil the growth is much more sparse. Wherever the third and fourth feet become dry in summer the plants are very small and scattered, leaving open spaces of bare soil. The seepweed is dormant during the winter, and a large part of the plant above ground dies. The new leaves and branches begin to show about March. The root system is not extensive, but usually extends through the third and fourth feet of soil (Pl. 7,A).

VARIATIONS FROM THE TYPICAL ASSOCIATION

Seepweed with Desert-sage

This mixture, the most important one of those formed by seepweed, occurs generally on the lower edge of the desert-sage lands. In many of these areas neither the seepweed nor the desert-sage are at their best. The land is too dry during certain periods of the year for a good growth of seepweed, while the desert-sage finds the salt content too high for the best growth. The salt content (Table XXIX) is higher than that of the desert-sage land but lower than that of the seepweed land.



TABLE XXIX.—Soil conditions in September and October in areas of seepweed mixed with other plants, Indio, Calif., 1914<sup>a</sup>

Item	Depth of soil	Date of collection				Average	Date of collection							Average	
		Sept. 23, sample No. 35, plant group D-Ap <sup>b</sup>	Oct. 2, sample No. 59, plant group D-Ap	Oct. 7, sample No. 64, plant group D-Ap	Oct. 13, sample No. 78, plant group D-Ap		Sept. 16, sample No. 20, plant group D-Ac	Sept. 19, sample No. 23, plant group D-Ac-Ap	Oct. 7, sample No. 67, plant group D-Ac-Ap	Oct. 14, sample No. 88, plant group D-P	Sept. 29, sample No. 51, plant group D-P	Oct. 1, sample No. 56, plant group D-P-Al	Oct. 15, sample No. 90, plant group D-Al		Oct. 14, sample No. 81, plant group D-I
Salt content.....	<i>Feet</i>														
	1	0.21	0.86	0.10	0.09	0.32	2.10	0.09	0.54	>2.50	2.30	>2.50	>2.50	1.10	1.40
	2	.20	.44	.07	.39	.28	0.56	.20	1.40	.24	.19	.80	.20	.72	.43
	3	1.25	.26	.17	1.18	.72	1.32	.24	2.08	.17	.08	.24	.08	.80	.52
Water content.....	4	.67	.30	.17	.98	.53	.74	.23	.70	.14	.07	.18	.18	.72	.27
	1	d <sup>c</sup>	d	d	d	-----	nd	d	d	w	w	w	w	w	d
	2	m	d	d	d	-----	sm	m	sm	w	w	w	w	w	sm
	3	d	d	d	nd	-----	sm	m	m	w	w	w	ss	w	m
Soil type.....	4	m	d	d	nd	-----	sm	m	m	w	w	w	sl	w	m
	1	s	fsl	fsl	sl	-----	fsl	s	s	fsl	sl	fsl	fsl	sl	l
	2	fsl	s	fsl	sl	-----	fsl	s	s	fsl	sl	fsl	sl	sl	s
	3	fsl	s	fsl	s	-----	l	s	s	sl	s	sl	sl	sl	s
	4	fsl	s	fsl	fsl	-----	l	s	s	s	s	sl	sl	s	s

<sup>a</sup> No soil moisture or moisture equivalent determinations were made on the 1914 soil samples. The data given are the field notes on the soil samples. Salt content data are stated in percentages of the dry weight of the soil.

<sup>b</sup> D = *Donardia torreyana* (S. Wats.) Standl.; Ap = *Atriplex polycarpa* (Torr.) S. Wats.; Ac = *Atriplex canescens* (Pursh) Nutt.; I = *Isocoma veneta acrademia* (Greene) H. M. Hall; P = *Pluchea sericea* (Nutt.) Coville; Al = *Atriplex lentiformis* (Torr.) S. Wats.

<sup>c</sup> d = dry soil; m = moist soil; sm = slightly moist soil; nd = nearly dry soil; w = wet soil; sa = saturated soil; s = sand and sandy; l = loam; fsl = fine sandy loam.

## Seepweed with Other Plants

Seepweed mixes with many other plants but these mixtures are of minor importance, covering only small areas. Table XXIX shows the salt content of the soil on which several of these mixtures occurred. The salt content is high wherever seepweed is found, with the exception of land formerly cultivated.

## ATRIPLEX FASCICULATA

The land covered by *Atriplex fasciculata* S. Wats. is similar in appearance and texture of soil to the "bare flats" or "slick land" discussed under the next heading. In fact, it is merely that part of the bare flats, usually only the outer edges in the larger bare flats, where the soil conditions have been made more favorable by the rains, that this *Atriplex* springs up. The communities formed by *Atriplex fasciculata*, although small, are pure. Only at the outer borders do they mix with *Atriplex linearis* S. Wats. and scattered *Lycium parviflorum* A. Gray. *Atriplex fasciculata* S. Wats. appears as a small annual from 6 to 10 inches high. It is often dense enough to cover the ground completely. This plant springs up after the rains when there is water in the surface foot on the flats, grows rapidly and as the water is exhausted the plants mature. Where sample No. 5, Table XXX, was collected, there is a heavy layer of silt on the surface that hinders penetration. There was no available water here in the fall. Sample No. 11, Table XXX, shows a lighter soil, better penetration, and some available water in the fall in all the feet except the first.

The salt content is high (Table XXX) with an appreciable quantity even in the first foot.

TABLE XXX.—A comparison of spring and fall soil conditions at the same station in a bare flat<sup>a</sup>

Item	Depth of soil	Chandler, Ariz., 1915, date of collection		Casa Grande, Ariz., 1915, date of collection	
		Mar. 25, sample No. 15	Oct. 7, sample No. 15	Oct. 11, sample No. 5 <sup>b</sup>	Oct. 11, sample No. 11 <sup>b</sup>
	<i>Feet</i>				
Moisture equivalent.....	1	20.1	19.7	22.9	17.7
	2	18.9	18.6	19.2	17.5
	3	16.0	18.2	12.3	17.1
	4	15.9	19.2	12.0	17.9
Wilting coefficient.....	1	10.9	10.7	12.4	9.6
	2	10.3	10.1	10.4	9.5
	3	8.4	9.9	6.7	9.3
	4	9.0	10.4	6.5	9.7
Moisture content above or below wilting coefficient...	1	-1.3	-4.4	c d	c d
	2	-0.5	0.0	d	sm
	3	+0.4	+1.2	d	sm
	4	+0.9	+2.9	d	sm
Salt content.....	1	2.12	2.68	.28	.34
	2	2.82	2.68	.48	1.00
	3	1.46	1.96	.32	.81
	4	1.00	1.58	.34	.81

<sup>a</sup> All data in percentages of the dry weight of the soil.

<sup>b</sup> Partly covered with an annual, *Atriplex fasciculata* S. Wats. No samples were taken here in spring.

c d=dry soil; sm=slightly moist soil.

## BARE FLATS

Scattered throughout the mesquite thickets and occasionally in the lower places in the desert-sage areas in the Gila Valley there are curious spots sometimes an acre or two in extent called "slick land," "bare flats," or "playa flats" (Pl. 14, B). The first name applies to the slippery nature of the heavy soil when wet, as in spring. The name "bare flats" refers to the lack of vegetation on these spots. The dry lakes of the Mohave Desert and Death Valley region would also be included. In spring, or after a heavy rain, water stands on these bare flats for some time. The soil is very heavy and silty on the surface, and when dried cracks, leaving deep fissures in the soil. On the most typical of these no plants grow. Occasionally there are scattered, small plants of *Dondia intermedia* (S. Wats.) Heller, *Atriplex polycarpa* (Torr.) S. Wats., and *Lycium parviflorum* A. Gray. These plants occurred on small hummocks of lighter soil which stand a foot or more above the level of the flat. After rains a small annual, *Atriplex fasciculata* S. Wats., springs up on the more favorable portions of the flat. A single station was chosen in a bare flat and a boring made in the spring and in the fall. Soil moisture is present in the third and fourth feet, probably indicating the influence of a high water table. Of the samples collected the first two feet had no available water (Table XXX). The salt content is very high, ranging over 2 per cent in the first and second feet. More than one-half of the salt content is made up of chlorids (Table II, sample No. 11).

## RELATIONS OF VEGETATION TO CLIMATIC CONDITIONS

The character of the vegetation in any region is an expression of the controlling factors of climate. The effect of moisture supply may be overcome or greatly altered by irrigation or by drainage. Temperature control is less likely to be locally interrupted except when there are abrupt variations in elevation. To express the temperature condition in a comparative way is very difficult, since summations are not yet possible, due largely to insufficient knowledge of plant physiology. We have not attempted the use of summation data, but have used the United States Weather Bureau summaries. These data are presented graphically in figures 7-9 and 14-21. In all cases the monthly values are plotted, beginning with January at the left. Rainfall is expressed in the usual way in figure 14. Below each graph is shown the mean annual rainfall, and the length of the record in years. The mean monthly temperature (fig. 15) is indicated by a bar and the lower extension drawn arbitrarily to 30 degrees below the annual mean value. This enables the reader to compare the different stations by a glance at the bottom of the mean monthly temperature graph. In figure 16 the absolute monthly maximum and the absolute monthly minimum are represented by the top and bottom of the light portion of the bars, while the mean monthly minimum and mean monthly maximum are represented by the bottom and the top of the shaded portion of the bar. On each graph are written the following annual values, the mean, the absolute maximum, the absolute minimum, the mean maximum and mean minimum, also the length of record in years, the type of vegetation, name of the station, and elevation above sea level.

The 41 stations shown in figure 14 are chosen to be fairly representative of the whole area of the southern desert, and of the types of vegetation considered in this paper. If we arrange these stations according to increasing rainfall, beginning with the lowest at the left, it is evident that the lowest rainfall occurs in those sections in which the vegetation is on the whole sparse and is composed either of creosote bush or desert-sage. All stations having a rainfall from 2 to 3½ inches are located in the Colorado desert of California. For the most part this rainfall occurs in late summer and there is little rain in early summer.

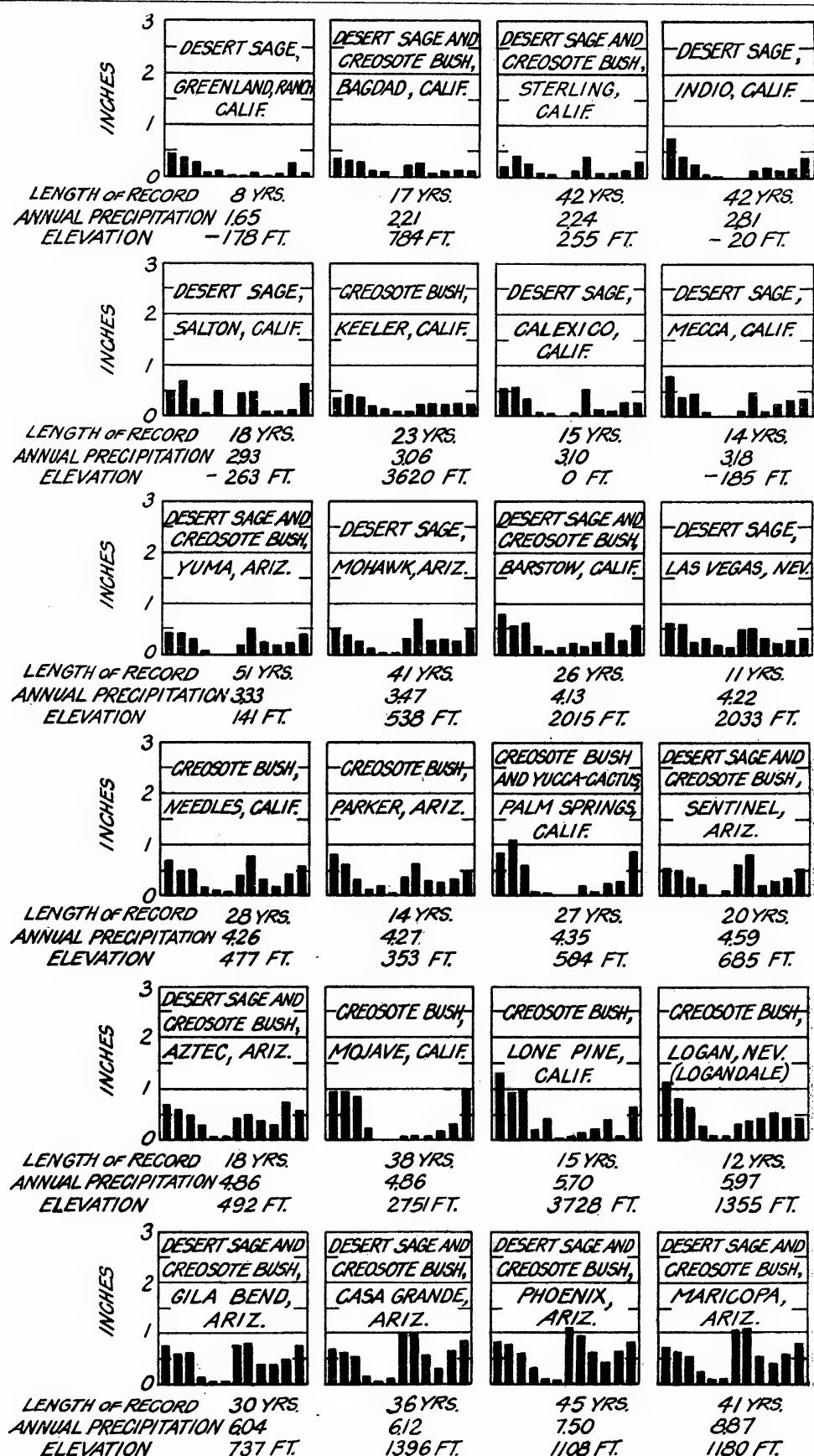
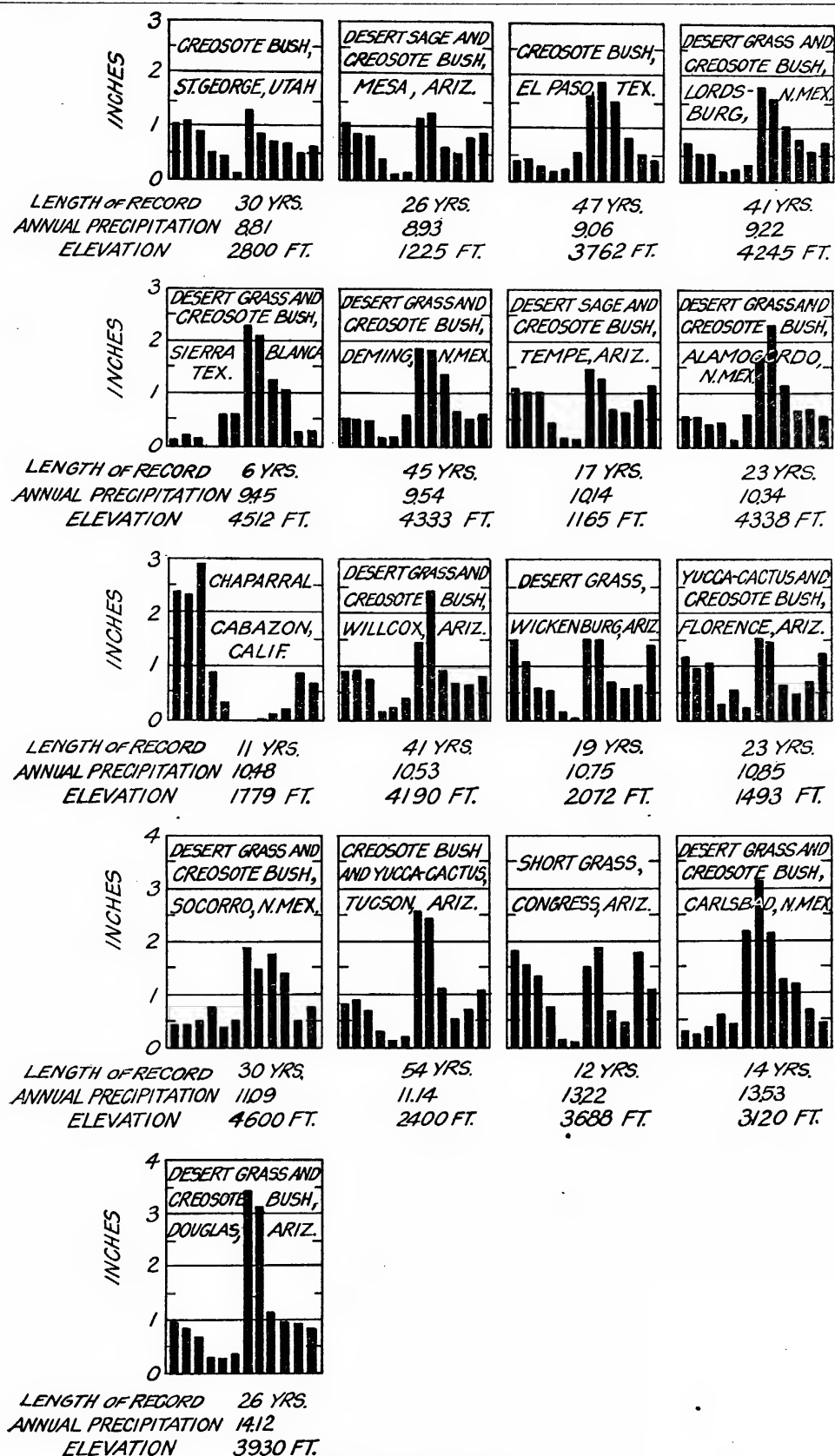


FIG. 14.—Monthly rainfall at 41 stations in the south desert shrub, arranged in order of increasing mean annual rainfall. The January rainfall is given in inches at the left of each small graph and the December



[Figure 14—Continued]

rainfall at the right. A prominent type of vegetation which occurs at the station and the name of the station are shown above the graph. Below it are given the mean annual precipitation, the length of the rainfall record in years, and the elevation above sea level.

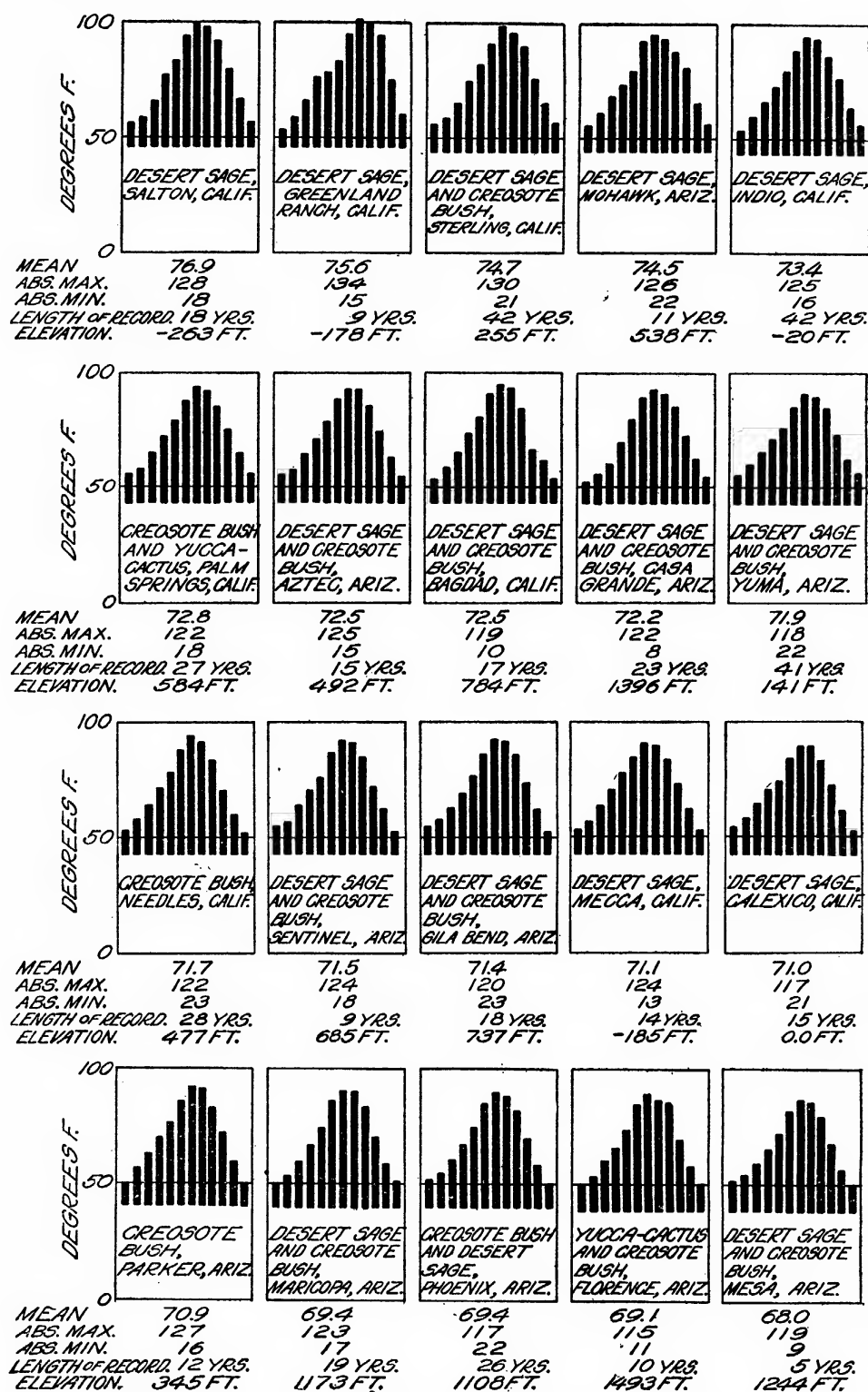
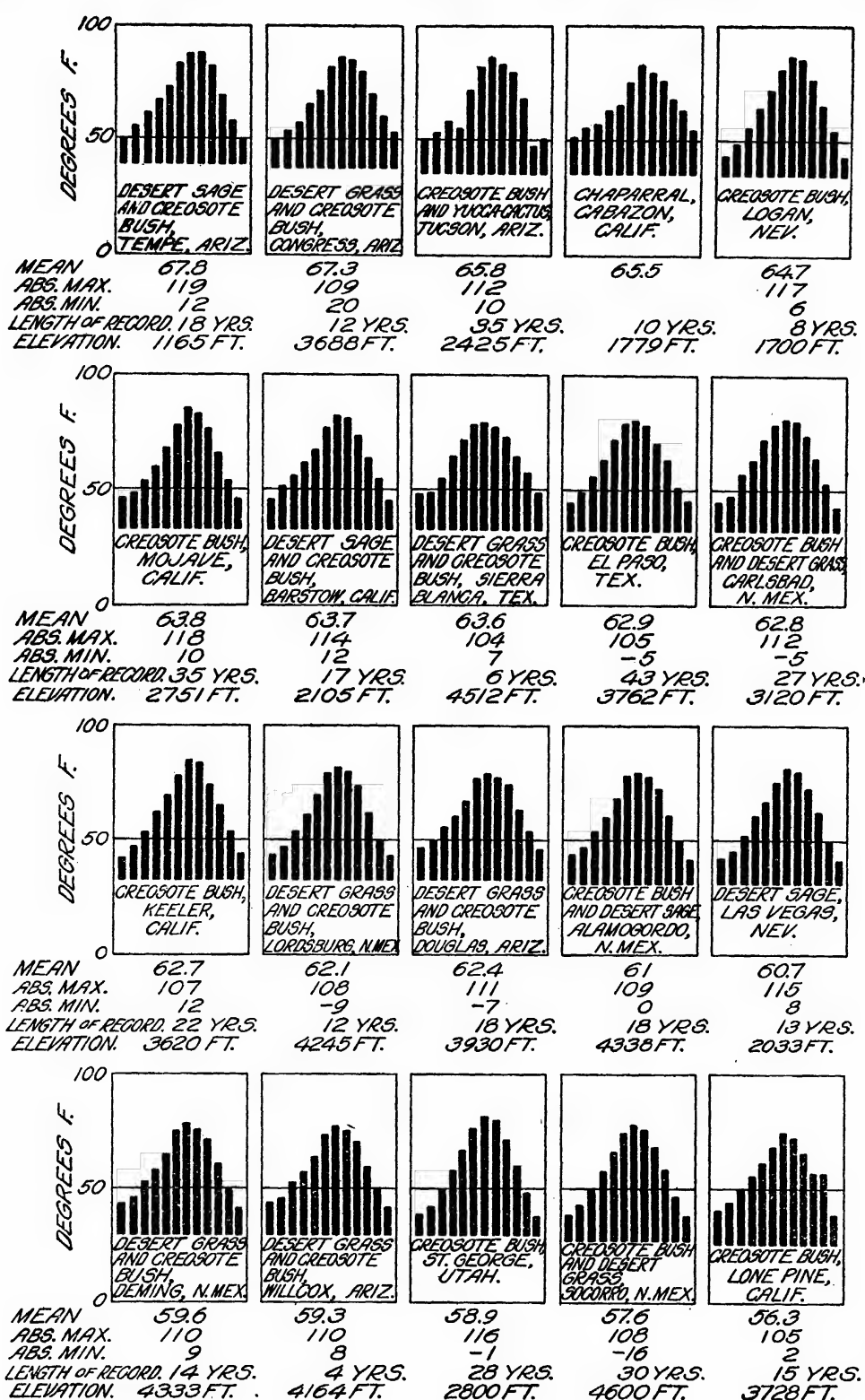


FIG. 15.—The mean monthly temperature of the 40 stations in the south desert shrub arranged in order of decreasing mean annual temperature, beginning with January at the left and ending with December at the right. To show at a glance the differences in the mean annual temperature the bars are cut off at the bottom at an arbitrary line 30 degrees below the mean annual temperature of the particular station.



[Figure 15—Continued]

In each graph are shown the type of vegetation and the station. Below in figures are given the mean, absolute maximum and absolute minimum temperatures, the length of the record in years and the elevation.

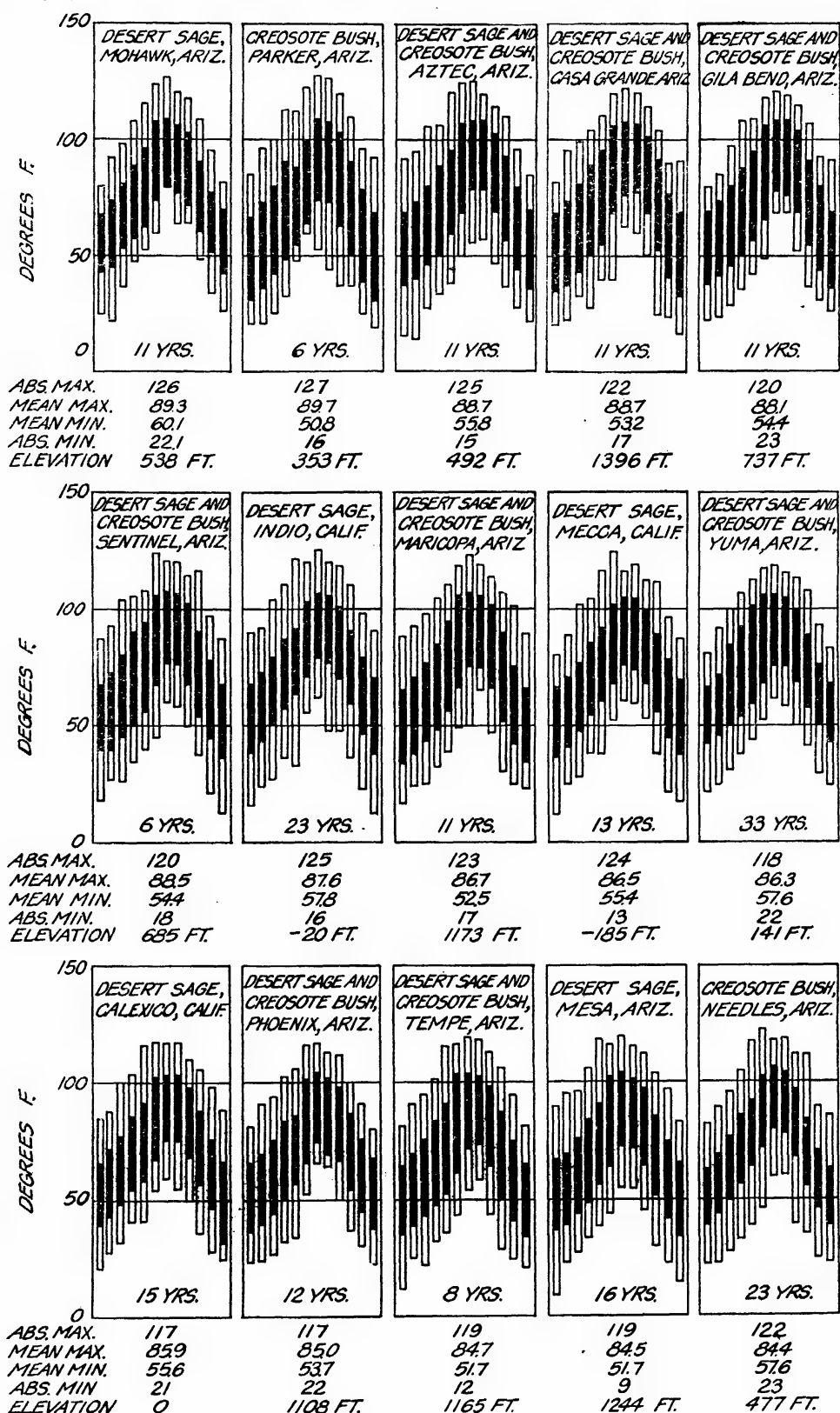
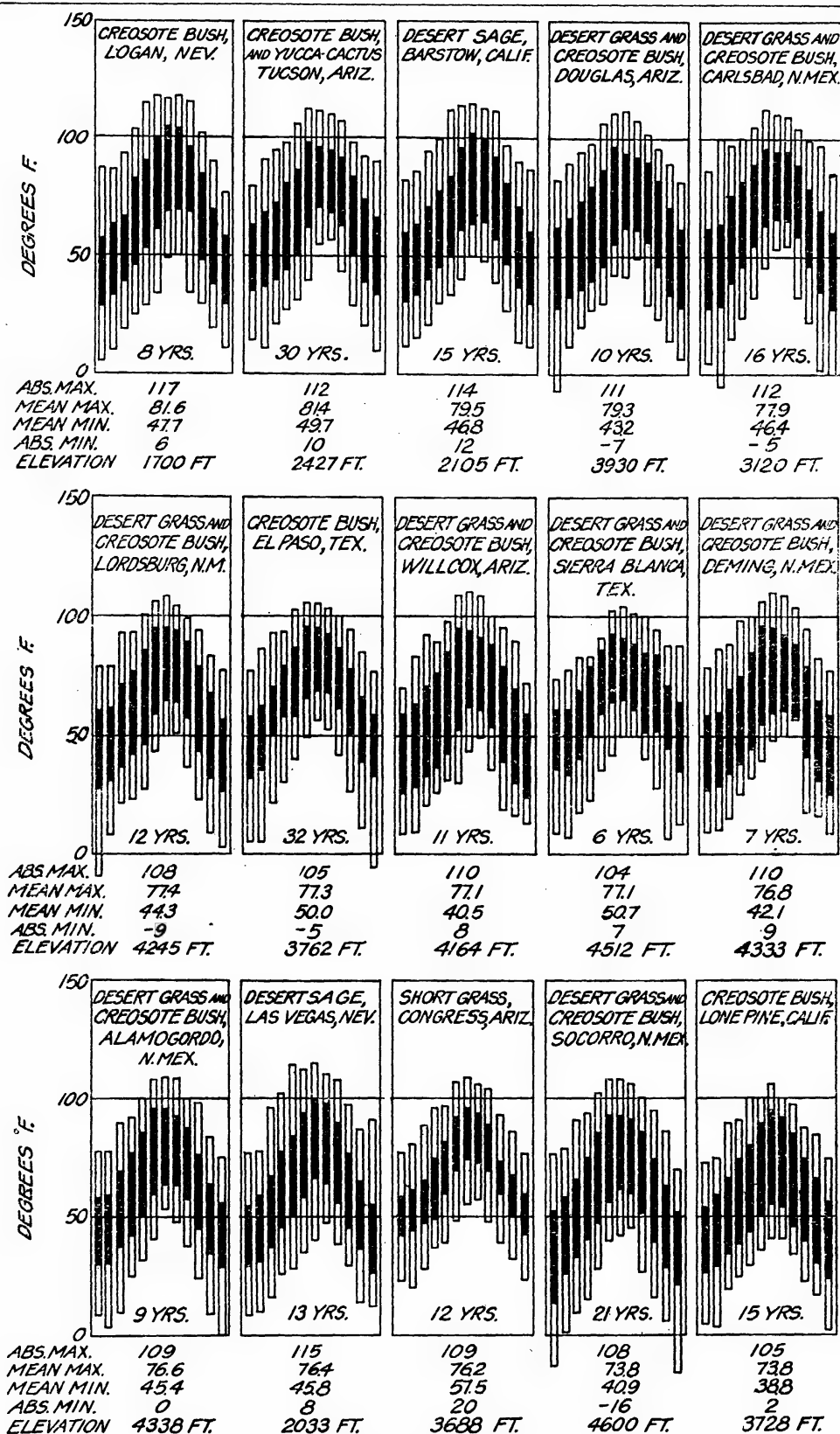


FIG. 16.—The monthly absolute maximum, absolute minimum, mean maximum, and mean minimum temperatures of 30 stations arranged in order of decreasing mean annual temperature. The absolute maximum and minimum for each month are shown by the upper and lower end of the light portion of the bar. The mean maximum and minimum by the upper and lower end of the shaded portion. The values for January are shown at the left, ending with December at the right. At the top within the graph is given





[Figure 16-Continued]

the predominating type of vegetation at the station followed by the name of the station. The period of years covered by the temperature records is written within the graph at the bottom. Below each graph are given the absolute maximum, mean maximum, mean minimum, and absolute minimum temperatures, and the elevation of the station in feet.

Those stations showing a rainfall between  $3\frac{1}{2}$  and 5 inches lie, for the most part in the Colorado desert, although two stations, Barstow and Mohave, lie in the portion known as the "Mohave desert," Greenland Ranch in Death Valley and Keeler in the Owens Valley. These are subject to a drought lasting at least through May and June. At a number of the stations a drought approximately six months long occurs during winter. Most of these stations, especially those in Arizona, show a tendency toward a rainy period during July and August; in other words, the summer rainy period so characteristic of the southern shrub desert, especially of New Mexico and Arizona, carries this influence beyond the Colorado river.

The stations showing a rainfall of between 5 and 9 inches are scattered throughout the area but usually occur at higher elevations than those of lower rainfall. In fact, there is rather strict correlation between elevation and rainfall in these stations. The vegetation shown in the region ranging from 2 to 9 inches is not markedly different in character but shows a correlation on the basis of amount or size of growth. The poorest growth, if washes and other locations which receive quantities of drainage water are eliminated, is found in the ranges which have a relatively low rainfall. At the stations showing from 5 to 9 inches of rainfall, the vegetation becomes much denser and more luxuriant, although the species may be the same as those showing a 2-inch rainfall.

Stations showing a rainfall of between 9 and 15 inches are quite often characterized by a grass cover in addition to a scattered growth of creosote bush or mesquite, though Cabazon with a rainfall of  $10\frac{1}{2}$  inches has a vegetation of chaparral, and Tucson and Florence with a rainfall of 12 inches have a particularly luxuriant type of desert shrub. Grasses are a prominent feature of the vegetation at practically all the other stations with a rainfall of 9 to 15 inches.

In general, then, we may say that the rainfall throughout the area characterized as southern desert varies from 2 to 15 inches and that where the rainfall is high, that is, approaching 15 inches, the vegetation is composed of large and luxuriant desert plants, or is characterized principally by grasses, but where the rainfall is less than 6 inches the vegetation is sparse and the plants are small.

If we consider mean temperature for the 40 stations (fig. 15) we find a correlation, in a general way, in that places receiving a low rainfall have, likewise, a high temperature.

The warmer stations are situated in the Colorado desert, the highest mean temperature, 77 degrees, being at Salton, Calif. Of the stations having a mean temperature above 70 degrees all are characterized by creosote bush, desert-sage, or salt desert vegetation. The other stations, which range from 60 degrees to 70 degrees in mean temperature are also characterized by a southern desert vegetation and, where moisture conditions are favorable, grasses often become a prominent feature. This is true at Congress, Sierra Blanca and, to some extent, at Carlsbad, Douglas, and Lordsburg. At Cabazon the mean annual temperature is 65 degrees but the high rainfall has favored the development of chaparral. The few remaining stations have a temperature ranging from 56 to 60 degrees. Willcox and Deming are characterized largely by grassland; St. George, Las Vegas, Socorro, and Lone Pine are stations characterized by both the northern and southern desert types of vegetation.

If we now consider the temperature maxima (fig. 16) we find that those stations which have an average maximum temperature of 85 degrees or above are limited largely to the Colorado desert. Of these all but three stations have absolute maxima of 120 degrees or above and four above 125 degrees. The monthly mean maximum is above 100 degrees for the four months, June, July, August, and September at nine of the stations considered. The stations having annual mean maximum temperatures between 80 degrees and 85 degrees either lie near the

northern edge of the great southern desert area, or they have a higher elevation. Of these five stations, all but one have absolute maxima above 115 degrees and the average monthly maxima exceed 100 degrees for three months in the year.

The remaining stations have an annual average maximum ranging from 74 to 80 degrees. In only one of these stations, Barstow, does the average maximum for any month reach 100 degrees, in fact only rarely does it reach 95 degrees. The highest absolute maximum in this region, 114 degrees, is at Barstow. All of these stations have recorded maximum temperatures of 100 degrees or more.

Considering the minimum temperature we find only six stations in the whole series fall to zero or below. No station, however, is frost free. An average minimum temperature of between 50 and 60 degrees is recorded for about half of the series, while the other half ranges from 39 to 50 degrees.

It is thus seen that the temperature conditions in much of the type of desert considered in this paper are relatively extreme. The plants growing in this desert are able to withstand excessively high temperatures but apparently can not push into any of the regions where the temperature falls far below zero. They appear to be able to withstand freezing temperatures, but even these low temperatures undoubtedly do much to change the character of the growth, since many of the more tender succulent plants can not well resist freezing. Creosote bush and mesquite are able to withstand temperatures of zero or below, provided, of course, these temperatures do not occur too often. Creosote bush and mesquite require relatively high temperatures during the growing period.

We may summarize briefly the extreme record for this desert type of vegetation. Desert-sage occurs in regions having a range of rainfall of from 2 to 12 inches and doubtless would flourish with a much higher rainfall. The average temperature ranges from 56 to 77 degrees, with average maxima ranging from 73 to 90.7 degrees and with absolute maxima ranging from 109 to 134 degrees. The minimum temperature for this plant ranges practically to zero. Creosote bush has a wider range than the desert-sage, since it occupies somewhat higher elevations and extends into regions of rather lower temperatures. Its range is the same as the total range for the southern desert shrub.

The areas in which the *Yuccas*, cacti, and other succulents are prominent do not include the hotter and drier stations of the lower land. These are also more limited by temperature so that the range of the succulents expressed roughly is from 4 to 12 inches in rainfall, from 63 to 73 degrees in average annual temperature, and, for the most part, below 85 degrees in average maximum temperature. Their absence from the hotter desert regions is probably more the result of the distribution of rainfall and the excessively long drought periods than the actual lower temperatures, since it is doubtful if the higher temperatures are effective in eliminating the succulents, if the distribution of rainfall is favorable.

A selected series of stations may now be considered in relation to vegetation and temperature and rainfall conditions (fig. 17). In passing eastward across the southern desert shrub, the conditions for growth become more favorable. Barstow is in the Mohave desert, where the vegetation is very sparse, and mostly of the creosote bush type. Casa Grande is in the Gila Valley and represents a much more luxuriant type of vegetation. Creosote bush, mesquite, and desert-sage are much more luxuriant than at Barstow. Tucson lies farther east and in a much more favorable region. It is located at the upper edge of the creosote bush zone and this plant is associated with a great profusion of desert forms, such as mesquite, giant cactus, paloverde and the *Opuntias*. It represents a luxuriant type of pure desert shrub. As one passes from Barstow to Tucson, the rainfall increases and the vegetation becomes more luxuriant. Barstow has a dry summer, from April to October, while both Casa Grande and Tucson have a drought period of only three months, April to June, inclusive.

As a result of this difference, Casa Grande and Tucson show a relatively great development of summer annuals. It seems probable that if the rainfall during August and September were increased somewhat, both places would develop a grass cover. Lordsburg is in a grass country with creosote bush a prominent plant. The grasses of the mesquite-grass type grow during the summer rainy period. The distribution of rainfall from July to December is similar to the Great Plains type, in a region intermediate between grassland and desert shrub. Lordsburg is somewhat cooler than the other stations considered, but moisture conditions chiefly are important in explaining this change in vegetation. Big Springs has the temperature required for the southern desert shrub and the rainfall sufficient to develop a grass cover. As a result, we have mesquite scattered over a mesquite-grass type. The range of temperature is a little greater at Big Springs and Lordsburg than at the other stations.

A similar series, but one which introduces temperature changes of significance, is shown in figure 18. Mohawk is located low in the Gila Valley. The temperature is very high, the mean annual being 74.5 degrees and the mean being above 90 degrees for three months of the year. During June, July, and August the mean maximum ranges from 105 degrees to 110 degrees and the absolute maximum reaches more than 100 degrees for seven of the twelve months of the year. The lowest temperature recorded is 22° F. Passing northeastward to Phoenix the rainfall becomes twice as heavy. Except for the more luxuriant growth, there is comparatively little change in vegetation. Creosote bush and desert-sage are the prominent types. The temperature is somewhat lower at Phoenix, but not low enough to eliminate any important element from the vegetation. At Socorro a marked change is noted in the vegetation. Creosote bush is near its limit, while the mesquite grassland and Yucca are prominent. Temperature falls to -16 degrees and the average is approximately 18 degrees lower than at Mohawk. The great change of temperature does not prevent the occurrence of mesquite and creosote bush. The rainfall is similar to Lordsburg, that is, of the Great Plains type, from July to November. Amarillo is in pure short-grass country. The average temperature is only a little lower than at Socorro. The summer rainfall is typical of the Great Plains and sufficient to develop a pure short-grass cover.

In this series, temperatures at Socorro and Amarillo are probably a little too low for the desert-shrub type, while the distribution of rainfall favors a grass cover. The short-grass cover would probably give way to mesquite grass if temperatures were as high at Amarillo as at Lordsburg or Big Springs, while Socorro would probably have the short-grass type instead of mesquite grass if the distribution of rainfall were similar to Amarillo.

A similar series selected in Arizona (fig. 19) shows a change from the luxuriant creosote bush type at Maricopa to the short grassland at Congress and to yellow pine at Williams. These changes are accompanied by temperature and precipitation differences. Maricopa and Congress do not show marked difference in temperature means, but Maricopa has a much higher summer maximum. Rainfall is twice as heavy at Congress as at Maricopa. This high rainfall favors the development of short-grass at Congress, while at Maricopa the grasses do not develop. At Williams, where the yellow pine is the dominant type, the rainfall is three times as heavy as at Maricopa, and the mean temperature about 20 degrees lower.

Minimum temperatures below zero are recorded only at Williams, and these low temperatures—the summer maximum seldom reaches 100 degrees—are in sharp contrast to Maricopa, where during four months of the year the average maximum exceeds 100 degrees and where zero temperatures are never recorded.

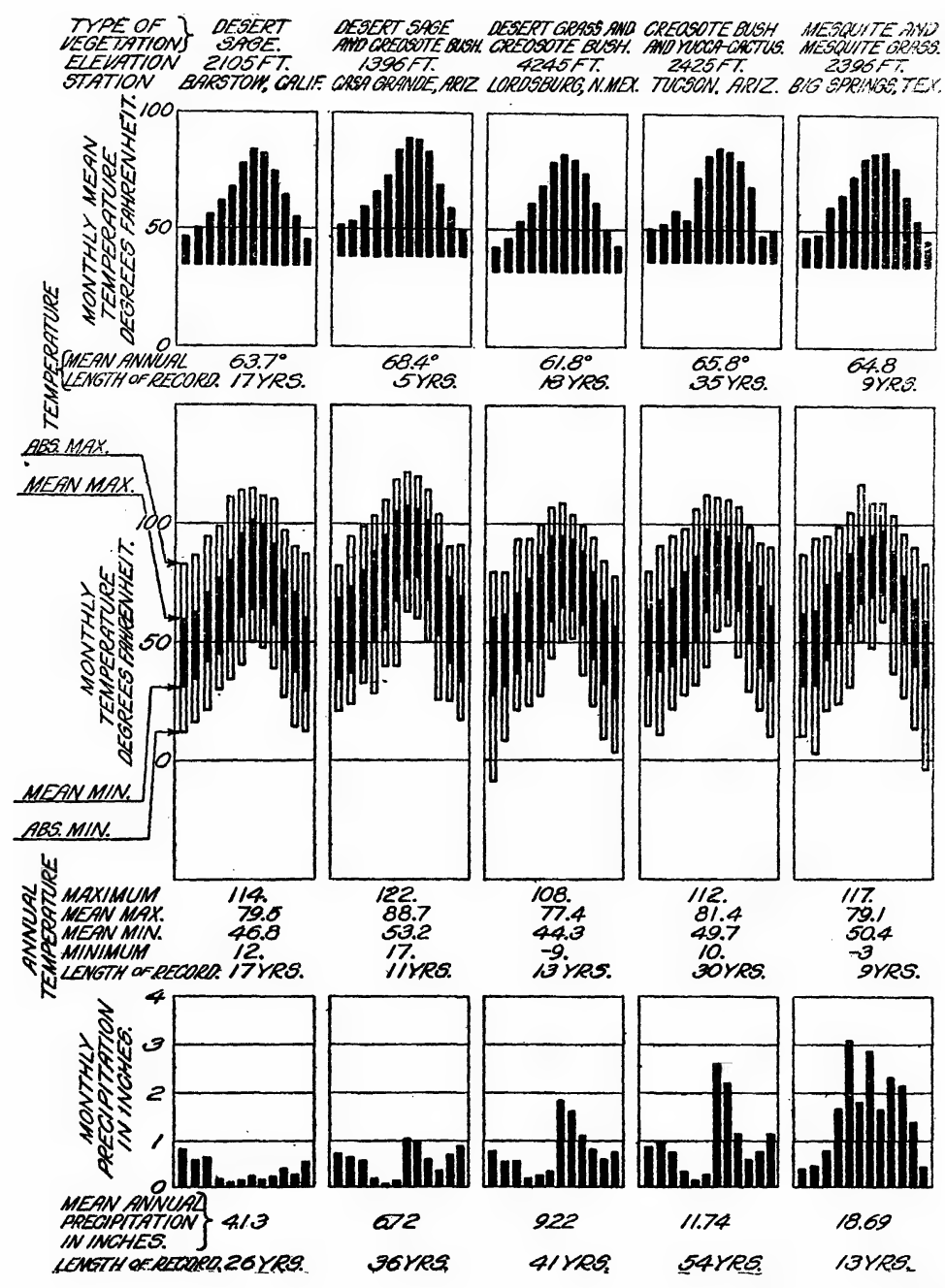


FIG. 17.—A comparison of vegetation with rainfall and temperature at Barstow, Calif.; Casa Grande and Tucson, Ariz.; Lordsburg, N. Mex.; and Big Springs, Tex. The graph shows monthly rainfall, monthly and annual mean, monthly absolute maximum and minimum, and monthly mean maximum and minimum temperatures for each station. In addition, the mean annual rainfall, the mean annual, the annual maximum, the mean maximum, the minimum, and the mean minimum temperatures, and the length of each record in years are given in figures below each graph. The type of vegetation and the name of the station with the elevation above sealevel are shown at the bottom of each column of graphs.

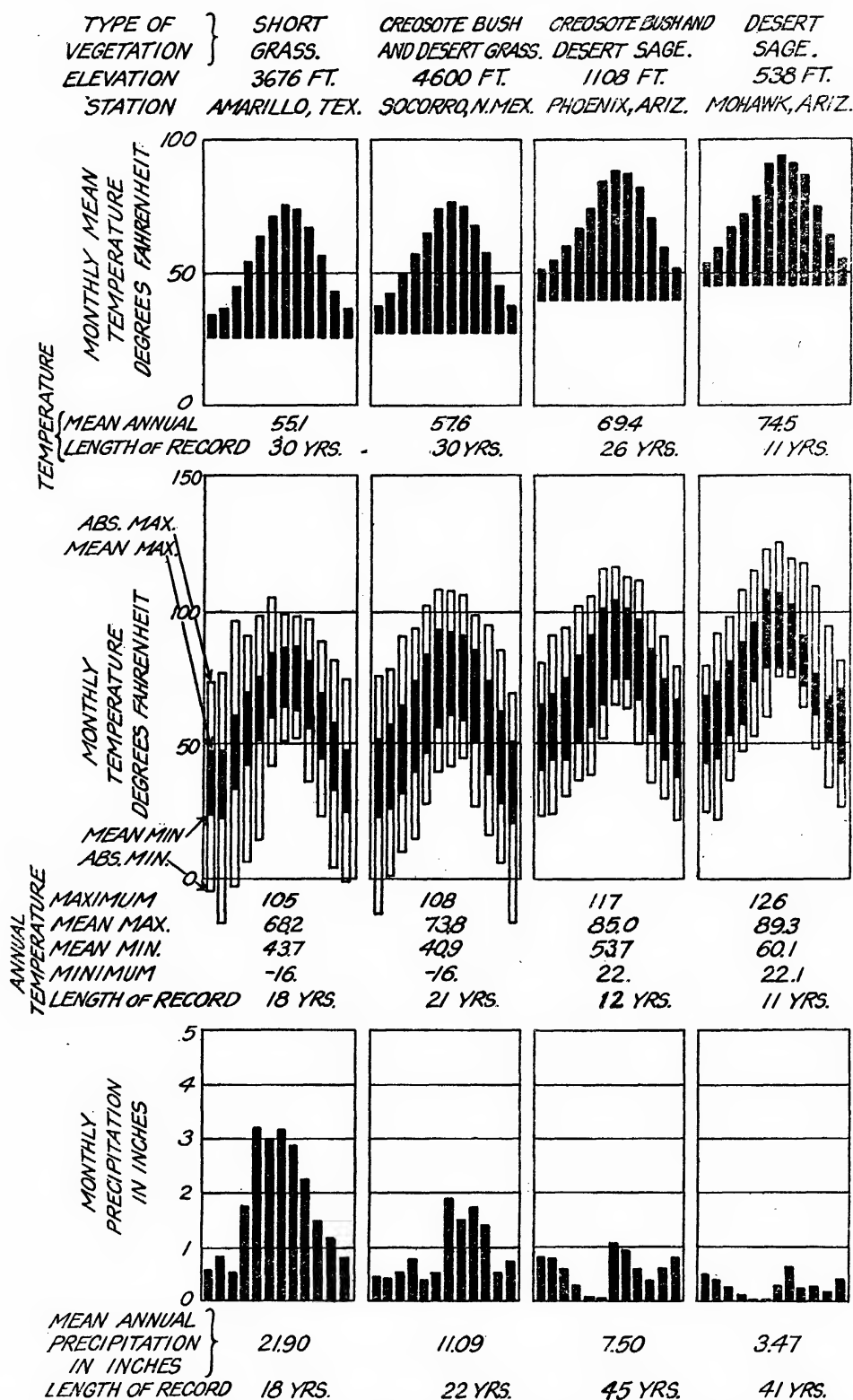


FIG. 18.—A comparison of the vegetation with rainfall and temperature at Mohawk and Phoenix, Ariz.; Socorro, N. Mex.; and Amarillo, Tex. The graph shows monthly rainfall, monthly and annual mean, monthly absolute maximum and minimum, and monthly mean maximum and minimum temperatures. In addition, the mean annual rainfall, the mean annual, the annual maximum, the mean maximum, the minimum and the mean minimum temperatures, and the length of each record in years are given in figures below each graph. The type of vegetation and the name of the station with the elevation above sealevel are shown at the bottom of each column of graphs.

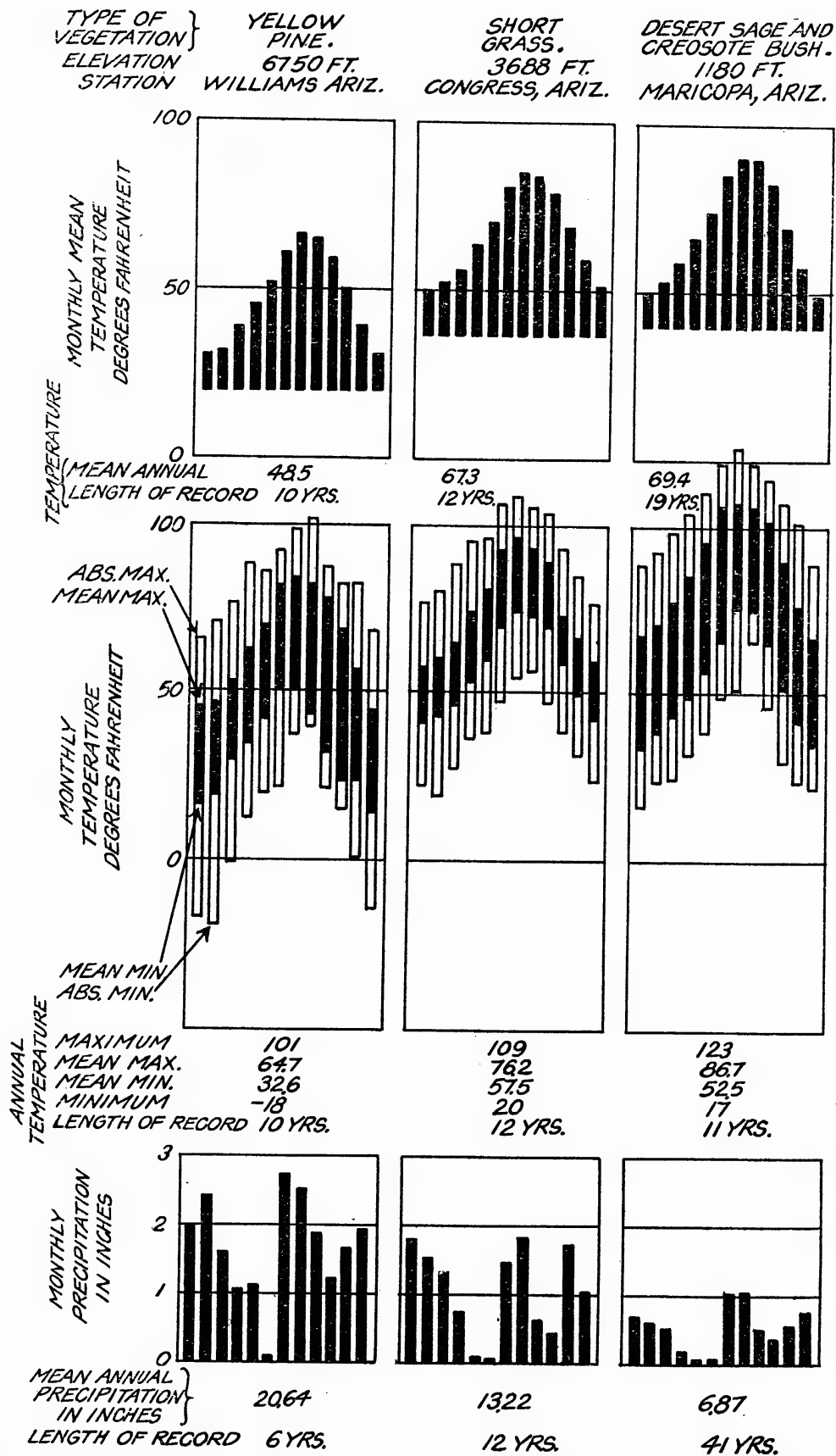


FIG. 19.—A comparison of the vegetation with rainfall and temperature at Maricopa, Congress, and Williams, Ariz. The graph shows monthly rainfall, monthly and annual mean, monthly absolute maximum and minimum, and monthly mean maximum and minimum temperatures. In addition, the mean annual rainfall, the mean annual, the annual maximum, the mean maximum, the minimum and the mean minimum temperatures, and the length of each record in years are given in figures below each graph. The type of vegetation and the name of the station with the elevation above sealevel are shown at the bottom of each column of graphs.

A similar series showing the effect of altitude may be selected ranging from Indio near the Salton Sink to Idyllwild on the San Jacinto Mountains (fig. 20). Palm Springs and Indio differ very slightly in temperature, but Palm Springs has a heavier rainfall. Neither is sufficient to change the type of vegetation. The slight differences that exist are due primarily to topography and soils. Rainfall at Cabazon is approximately three times as heavy as at Indio and Palm Springs and the temperature about 8 degrees lower. The vegetation at Cabazon is of the chaparral type. Proceeding to the higher altitude of the yellow pine zone at Idyllwild, the rainfall is about ten times as heavy as at Palm Springs. The temperature is much lower, the average being about 52 degrees as compared with 65 degrees in the chaparral and 73 degrees in the creosote bush zone, although the temperatures do not fall below zero. The difference in average mean temperature between the lower creosote bush zone and the yellow pine zone is approximately the same as between the same types at Maricopa and Williams.

A similar series ranging from the Colorado desert northward (fig. 21) has been chosen. In this series, Indio represents vegetation consisting largely of desert-sage, although the climate is not essentially different in the creosote bush areas at a somewhat higher elevation. Las Vegas, Nev., has a vegetation composed of desert-sage and creosote bush at a higher elevation, with an admixture of shadscale. Therefore, Las Vegas may be regarded as near the northern limit of the southern desert. St. George, Utah, shows a vegetation characteristic of the northern desert, and represents almost the northern limit of the southern desert vegetation. At Parowan, Utah, the vegetation is typical of that of the northern desert. It consists of a great sagebrush area lying near the lower border of the juniper-piñon belt. Still another station, Yellowstone, may be chosen to represent conditions of sagebrush where it occurs close to the yellow pine zone.

In comparing the rainfall of these five stations we find that Indio has a rainfall of less than 3 inches, and Las Vegas less than  $4\frac{1}{2}$  inches, St. George has 9 inches, Parowan 13 inches, and Yellowstone Park 18 inches. On the conditions of moisture supply, therefore, the conditions improve as we pass northward, where there is also increase in elevation. Indio has an elevation of minus 20 feet, Las Vegas a little over 2,000 feet, St. George less than 3,000 feet, Parowan about 6,000 feet, and Yellowstone 6,200 feet, a difference of more than 6,000 feet. Rainfall is more uniform at Yellowstone throughout the season, and it is the only station which has over  $\frac{1}{2}$  inch precipitation during June. The temperature conditions are markedly different in these five stations. Indio has an average temperature of 73 degrees, Las Vegas 61 degrees, St. George 59 degrees, Parowan 49 degrees, and Yellowstone Park 39 degrees. These differences in mean temperature are also accompanied by the similar differences in the maximum and minimum temperatures. The lowest at Indio is 16 degrees, at Las Vegas 8 degrees, at St. George -1 degree, at Parowan -18 degrees, and at Yellowstone Park -41 degrees. Parowan and Yellowstone Park represent conditions in the northern desert area, therefore much colder, and have a much greater and more equally distributed rainfall than any of the stations in the southern desert shrub.

On the basis of yearly summaries, the evaporation rate in this southwestern desert is high and the depression of the wet bulb is also high.<sup>8</sup> However, if only the period of growth is considered the conditions are by no means extreme. Briggs and Shantz<sup>9</sup> have found a close correlation between rate of transpiration

<sup>8</sup> KINCER, J. B. PRECIPITATION AND HUMIDITY. *Atlas of American Agriculture*, Part II, Section A, Fig. 103-105. 1922.

<sup>9</sup> BRIGGS, L. J., and SHANTZ, H. L. HOURLY TRANSPIRATION RATE ON CLEAR DAYS AS DETERMINED BY CYCLIC ENVIRONMENTAL FACTORS. *Jour. Agr. Research* 5:583-650, illus. 1916.

———. DAILY TRANSPIRATION DURING THE NORMAL GROWTH PERIOD AND ITS CORRELATION WITH THE WEATHER. *Jour. Agr. Research* 7:155-212, illus. 1916.



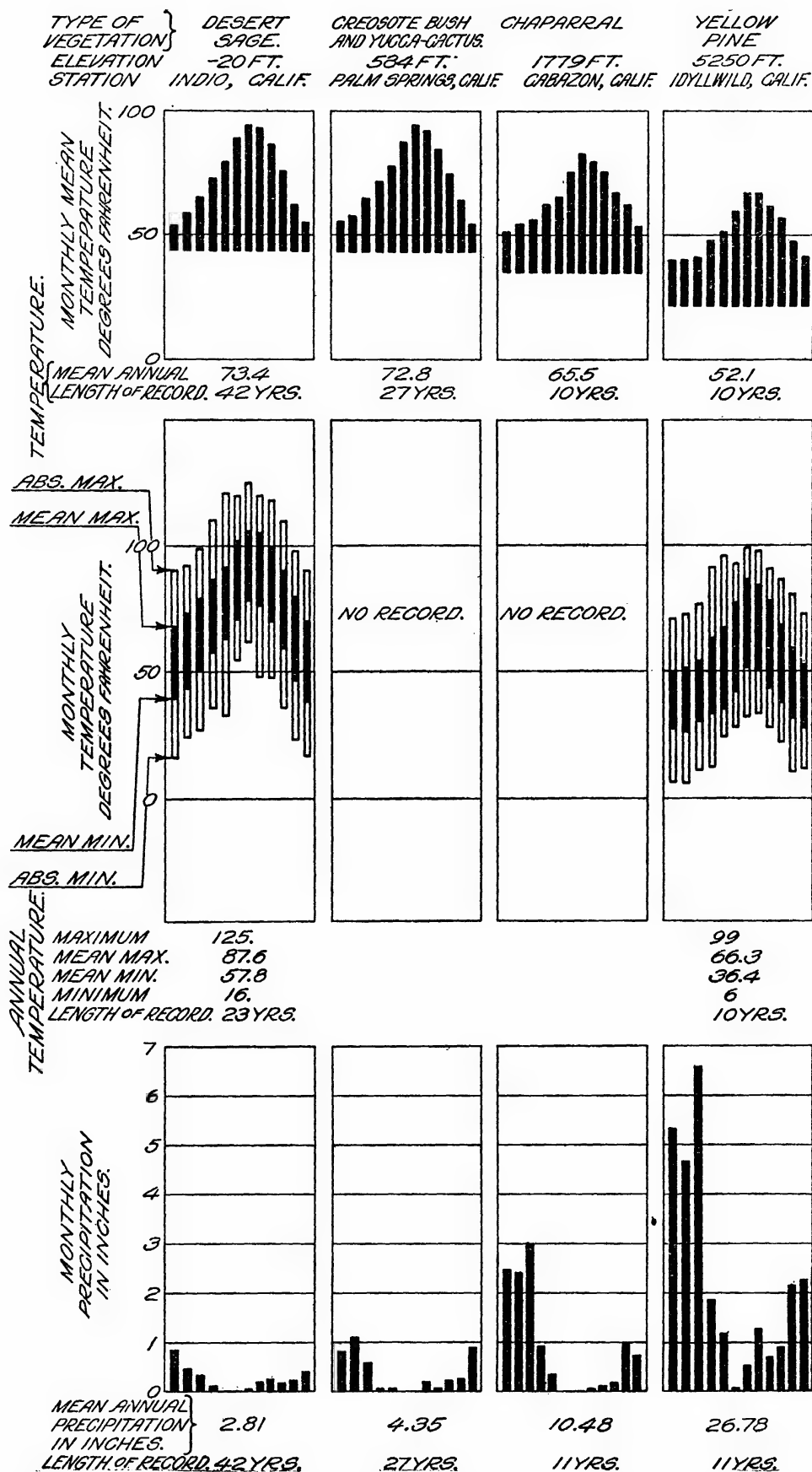


FIG. 20.—A comparison of the vegetation with rainfall and temperature at Indio, Palm Springs, Cabazon, and Idyllwild, Calif. The graph shows monthly rainfall, monthly and annual mean, monthly absolute maximum and minimum, and monthly mean maximum and minimum temperatures. In addition, the mean annual rainfall, the mean annual, the annual maximum, the mean maximum and minimum, and the mean minimum temperatures, and the length of each record in years are given in figures below each graph. The type of vegetation and the name of the station with the elevation above sealevel are shown at the bottom of each column of graphs.

and radiation and wet bulb depression. On this basis the transpiration during the summer season should be very high in this desert if plants were in an actively growing condition. This, however, is not the case, since practically all plant growth is made during the late winter and early spring months. If comparisons are made between conditions at Akron, Colo., and Bard, Calif., during the period

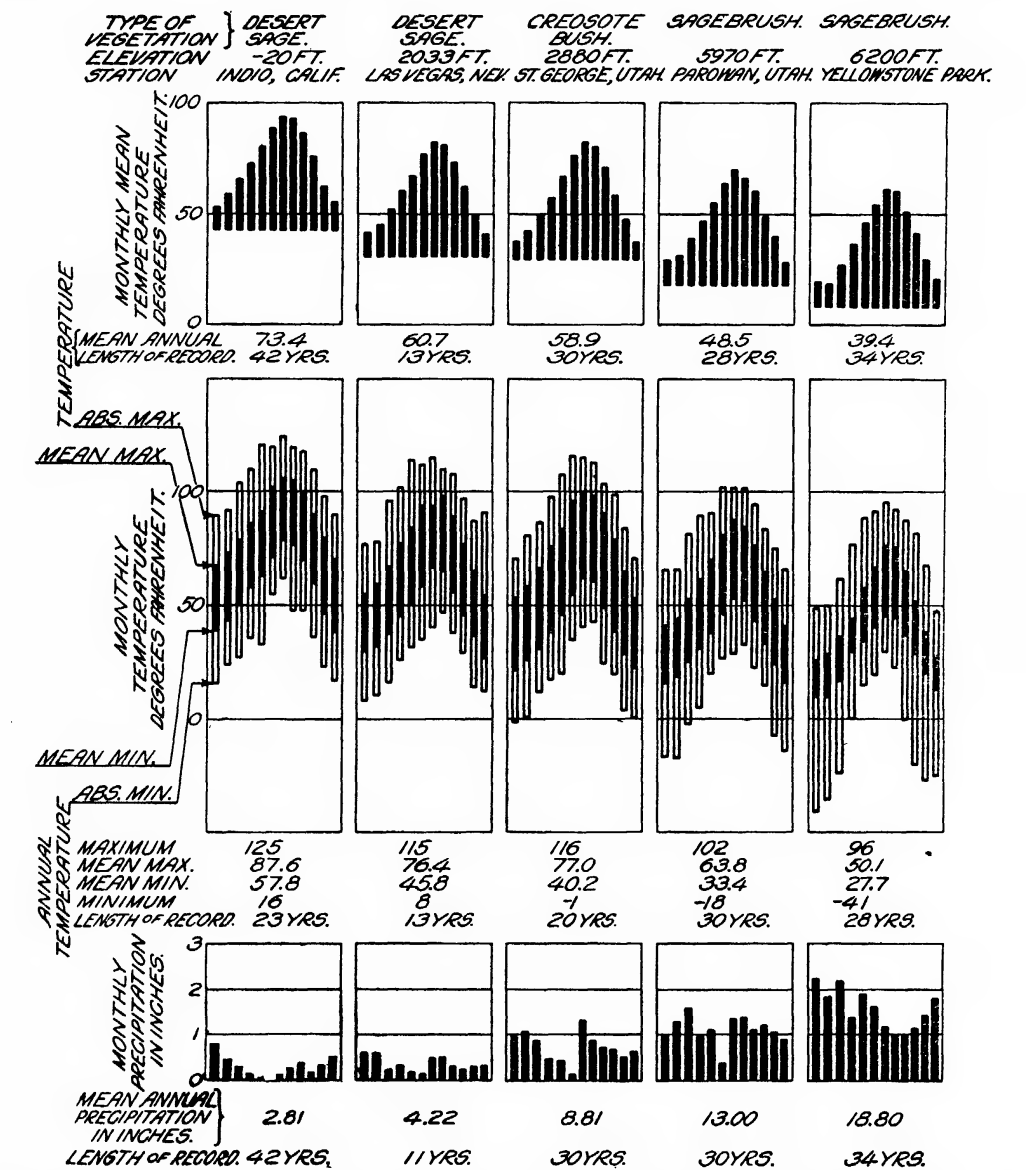


FIG. 21.—A comparison of the vegetation with rainfall and temperature at Indio, Calif., Las Vegas, Nev., St. George and Parowan, Utah, and Yellowstone, Wyo. The graph shows monthly rainfall, monthly and annual mean, monthly absolute maximum and minimum, and monthly mean maximum and minimum temperatures. In addition, the mean annual rainfall, the mean annual, the annual maximum, the mean maximum, the minimum and the mean minimum temperatures, and the length of each record in years are given in figures below each graph. The type of vegetation and the name of the station with the elevation above sealevel are shown at the bottom of each column of graphs.

of growth it is evident that the plant growth in this desert region is not subjected to conditions as extreme as at Akron, Colo. The months January, February, and March at Bard correspond as regards the time of active growth with May, June, and July at Akron, Colo. During these periods the light intensity<sup>10</sup> at Bard is

<sup>10</sup> KIMBALL, H. H. VARIATIONS IN THE TOTAL AND LUMINOUS SOLAR RADIATION WITH GEOGRAPHICAL POSITION IN THE UNITED STATES. Mo. Weather Rev. 47:769-793, illus. 1919.

between 300 and 500 gm. calories per sq. cm. per minute, while at Akron it is between 600 and 650. The evaporation from a 6-foot tank of water at Bard for January to March, inclusive, for the nine-year period, 1911-1919, averaged 12 inches, while at Akron for May to July, inclusive, for the thirteen-year period, 1908-1920, averaged 24 inches. A comparison of the water requirement of small grains at Akron and at Bard shows the requirements at Akron to be about 25 per cent higher than at Bard.

It is therefore evident that, in the Colorado desert, during the most active growing period, the conditions favoring a rapid transpiration are not so extreme as they are during the most active growing period in the central high plains.

## CORRELATION BETWEEN THE TYPES OF VEGETATION AND THE CHARACTER AND PRODUCTIVITY OF THE LAND

### CORRELATION WITH PHYSICAL CONDITIONS

While each of the valleys of the Southwestern desert region wherein studies were made differ somewhat in vegetation, the most important types are common to all of them. These types indicate fairly definite soil conditions. There is, however, considerable variation and the correct interpretation of the types of vegetation is often difficult. The wide variation in soil conditions where some of the types occur makes it necessary to know more than merely the fact that a certain type of vegetation is present on a piece of land. For instance, creosote bush throughout the southwestern desert region indicates well-drained, nonsaline land with a low water table. Whether the soil is of good depth or shallow is understood much better if it is known whether the growth of the creosote bush is tall and vigorous or scrubby and poor. Likewise, if the creosote bush is mixed with the bur-sage or if bur-sage occurs near by, the soil is likely to be looser, stonier and less fertile. If the creosote bush is mixed with desert-sage or occurs near the desert-sage area the soil conditions indicated are a finer, more fertile soil not so permeable and possibly with a trace of salinity in the subsoil. Creosote bush in regions of higher rainfall as in eastern Arizona is often characteristic of land with caliche near the surface.

The physical condition of the soil characterized by each type of vegetation is summarized in Tables XXXI and XXXII. The results of study of these tables are given in Table XXXIII which summarizes the conditions under each type of vegetation. In Table XXXIV a summary is given of salinity of the soils at different depths under the different types of vegetation and the different stations. The results of a study of these tables were included in the following general summary of the conditions under each type.

#### CREOSOTE BUSH (*COVILLEA GLUTINOSA* (ENGELM.) RYDB.)

While creosote bush land is usually porous, nonsaline and high above the water table, it is not always good farming land. That which is covered by a good growth of creosote bush, about 5 feet or higher, is good farming land having a good depth of porous nonsaline and well-drained soil. That which is covered with a poor, scrubby growth, usually less than 4 feet high, is land either too stony or else too shallow, due to underlying hardpan or rock layers, to be farmed. At the present time much of the creosote bush land is uncultivated, for, as a rule, it lies above the highest canals that supply the irrigation water. These lands, composing as they do the warmest slopes of the valleys, are probably best adapted to fruit culture.

TABLE XXXI.—Comparison of the spring and fall soil conditions at the same stations in the principal plant communities at Indio, Calif., 1915 <sup>a</sup>

Item	Depth of soil	Plant community																				
		Creosote bush			Desert-sage			Mesquite and chamiso			Seepweed			Saltbush and arrow-weed			Pickleweed			Saltgrass		
		Spring	Fall	Average	Spring	Fall	Average	Spring	Fall	Average	Spring	Fall	Average	Spring	Fall	Average	Spring	Fall	Average	Spring	Fall	Average
Moisture equivalent.....	Feet																					
	1.....	3.3	3.7	3.5	24.0	24.2	24.1	2.3	2.6	2.5	23.5	27.1	25.3	28.1	23.1	20.6	21.9	27.1	29.7	28.4	27.1	29.7
	2.....	3.5	4.5	4.0	25.9	26.3	26.1	2.7	2.8	2.8	26.9	28.0	27.5	19.6	23.1	23.2	23.2	21.3	20.3	20.8	21.3	20.3
	3.....	2.8	3.9	3.4	23.0	23.5	23.5	2.6	2.8	2.7	20.7	19.8	20.3	16.0	18.2	18.1	18.2	35.5	30.1	32.8	35.5	30.1
Wilting coefficient.....	4.....	2.7	4.1	3.4	19.1	22.4	20.8	2.8	3.1	3.0	25.3	23.9	24.6	13.6	17.0	10.4	13.7	22.5	21.0	21.8	22.5	21.0
	1.....	1.8	2.0	1.9	13.1	13.2	13.2	1.3	1.4	1.4	12.7	14.7	13.7	15.3	12.6	11.2	11.9	14.7	16.2	15.4	14.7	16.2
	2.....	1.9	2.5	2.2	14.1	14.3	14.2	1.5	1.6	1.6	14.6	15.2	14.9	10.7	12.5	12.7	12.6	11.6	11.0	11.3	11.6	11.0
	3.....	1.5	2.1	1.8	12.6	13.0	12.8	1.4	1.5	1.5	11.3	10.7	11.0	8.7	9.9	9.8	9.9	19.3	16.4	17.9	19.3	16.4
Moisture content above or below wilting coefficient.	4.....	1.5	2.2	1.9	10.4	12.2	11.3	1.5	1.7	1.6	13.8	13.0	13.4	7.4	9.2	5.6	7.4	12.3	11.4	11.9	12.3	11.4
	1.....	3.0	1.2	---	3.3	10.2	---	2.4	1.3	---	5.7	10.8	---	14.1	17.5	5.8	---	16.3	5.4	---	16.3	5.4
	2.....	4.4	1.6	---	2.9	8.5	---	3.8	1.0	---	8.2	5.7	---	12.5	16.1	9.0	---	17.4	9.8	---	17.4	9.8
	3.....	4.4	1.4	---	4.2	6.6	---	4.1	0.3	---	4.2	0.1	---	12.0	18.4	13.2	---	19.1	14.3	---	19.1	14.3
Salt content.....	4.....	2.3	1.3	---	5.1	6.6	---	0.4	0.1	---	2.3	0.6	---	16.4	20.8	12.0	---	19.4	17.7	---	19.4	17.7
	Average.	3.5	1.4	---	2.2	8.0	---	2.7	0.7	---	5.1	3.9	---	13.8	19.1	10.0	---	17.6	11.8	---	17.6	11.8
	1.....	.01	.01	.01	.26	.25	.26	.01	.01	.01	.99	1.09	1.04	2.32	2.50	2.50	2.50	2.45	2.08	2.27	2.45	2.08
	2.....	.01	.01	.01	.70	.40	.55	.01	.01	.01	.69	1.05	.87	.63	1.86	1.38	1.62	1.32	1.32	1.30	1.32	1.30
Average.	3.....	.01	.01	.01	.32	.22	.27	.02	.01	.02	.50	.95	.73	.28	1.17	.92	1.05	.36	1.03	1.70	.36	1.03
	4.....	.01	.01	.01	.27	.21	.24	.02	.04	.03	.78	.75	.77	.20	.48	.44	.46	.20	.53	.70	.20	.53
	Average.	.01	.01	.01	.39	.27	.33	.02	.02	.02	.77	.96	.87	.90	1.50	1.31	1.41	1.07	1.24	1.16	1.07	1.24



TABLE XXXIII.—A summary of the soil conditions as indicated by the principal plant communities of the Southwestern Desert

Types of vegetation	Condition of vegetation	Kind of soil		Moisture		Salinity	
		Surface foot	Subsoil	Surface foot	Subsoil	Surface foot	Subsoil
Cresote bush	Good growth. Poor growth.	Sandy loam. Often stony or if as above (see under subsoil).	Sandy loam. Hardpan or rock layer more rarely alkali.	Moist after rains only. do.	Moist after rains only. Apt to be dry due to lack of penetration.	Nonsaline. do.	Nonsaline. Rarely slightly saline.
Desert-sage	Good growth. Poor growth.	Fine sandy loam. do.	Fine sandy loam. Hardpan or alkali.	do. do.	Moist after rains only. Apt to be dry due to lack of penetration.	do. do.	Nonsaline. Saline.
Mesquite and chamiso.		Sand.	Sand.	do.	Moist after rains only	do.	Nonsaline.
Chamiso.	Rank growth.	Sandy.	Sandy.	do.	do.	do.	Do.
Seepweed		Fine sandy loam.	Fine sandy loam.	Moist <sup>a</sup> in spring. Rains and high water table.	Moist <sup>a</sup> most of year. High water table.	Saline <sup>a</sup> .	Saline. <sup>a</sup>
	Poor growth.	do.	do.	Moist only after rains.	Moist part of year. Rains and high water table.	do.	Do.
Saltbush and arrow-weed.		Loam.	Fine sandy loam with sand layers.	Moist most of year. High water table.	Moist all the year.	Strongly saline.	Moderately saline.
Pickweed		Fine sandy loam to loam.	do.	Moist all the year. High water table.	High water table. Wet all the year. High water table.	do.	Strongly saline.
Saltgrass		Clay loam, fine sandy loam, sand and gravel, etc.	Clay loam, fine sandy loam, sand and gravel, etc.	do.	do.	do.	Do.
Mesquite thicket		Clay loam.	Clay loam.	Moist after rains only.	Moist all the year. High water table.	Slightly saline.	Do.
Saltbush		Heavy clay loam.	Heavy clay loam.	Wet most of the year. High water table.	Wet all the year. High water table.	Saline.	Saline.

<sup>a</sup> Except where it is a weed, as on an abandoned field; then a rank growth may indicate nonsaline soil and temporary moisture.

TABLE XXXIV.—Salt content of the soils occupied by the principal plant communities of the Southwestern Desert <sup>a</sup>

Depth of soil	Creosote bush ( <i>Corillea glutinosa</i> )				Desert-sage ( <i>Atriplex polycarpa</i> )					Seepweed ( <i>Dondia torreyana</i> and <i>D. intermedia</i> )				Mesquite and chami-so ( <i>Prosopis glandulosa</i> - <i>Atriplex canescens</i> )			
	Indio, 1915, 10 samples	Chandler, 11 samples	Casa Grande, 4 samples	Average	Indio, 1914, 26 samples	Indio, 1915, 10 samples	Chandler, 16 samples	Casa Grande, 3 samples	Average	Indio, 1914, 9 samples	Indio, 1915, 6 samples	Casa Grande, 1 sample	Average	Indio, 1915, 10 samples	Chandler, 8 samples	St. George, 1 sample	Average
<i>Feet</i>																	
1-----	0.01	0.05	0.02	0.03	0.30	0.26	0.06	0.04	0.17	1.55	1.04	0.25	0.95	0.01	0.02	0.12	0.05
2-----	.01	.04	.02	.02	.45	.55	.23	.07	.33	1.25	.87	.50	.87	.01	.06	.12	.06
3-----	.01	.05	.03	.03	.33	.27	.35	.08	.26	.89	.73	1.04	.89	.02	.08	.14	.08
4-----	.01	.08	.05	.05	.31	.24	.42	.14	.28	.77	.77	.70	.75	.03	.16	.19	.13
Average....	.01	.06	.03	.03	.35	.33	.27	.08	.26	1.12	.87	.62	.90	.02	.08	.14	.08

Depth of soil	Saltbush and arrow-weed ( <i>Atriplex lentiformis</i> - <i>Pluchea sericea</i> )			Saltbush ( <i>Atriplex lentiformis</i> )			Pickleweed ( <i>Allenrolfea occidentalis</i> )			Saltgrass ( <i>Distichlis spicata</i> )		
	Indio, 1914, 2 samples	Indio, 1915, 6 samples	Average	Indio, 1914, 2 samples	Chandler, 6 samples	Average	Indio, 1914, 7 samples	Indio, 1915, 6 samples	Average	Indio, 1914, 3 samples	Indio, 1915, 4 samples	Average
<i>Feet</i>												
1-----	1.19	2.32	1.76	>2.50	0.76	1.63	1.78	2.50	2.14	1.80	2.27	2.04
2-----	.66	.99	.83	.44	.62	.53	1.46	1.64	1.55	1.23	1.30	1.27
2-----	.66	.39	.53	.22	.47	.35	1.21	1.05	1.13	.94	.70	.82
4-----	.70	.35	.53	.12	.61	.37	.78	.46	.62	.69	.37	.53
Average.....	.80	1.01	.91	.82	.62	.72	1.31	1.41	1.38	1.17	1.16	1.17

<sup>a</sup> All data in this table are stated in percentages of the dry weight of the soil.

DESERT-SAGE (ATRIPLEX POLYCARPA (TORR.) S. WATS.)

Probably the largest part of the land now under irrigation in the Coachella and Gila Valleys is desert-sage land. While this land is usually slightly saline, with irrigation the amount of salt is not prohibitive. Here, as in the case of creosote bush land, the condition of the desert-sage means much. Where there is a pure good growth of desert-sage the soil is a nonsaline sandy loam, considerably above the water table. Where the growth is poor, however, the salinity increases considerably and, in some cases, there is a hardpan. Though the saline content is rarely high enough to be serious, yet the silty layers of soil, or sometimes the hardpan, may make it difficult to remove the salts by leaching. Both fruits and alfalfa are grown on this type of land to a large extent.

SEEPWEED (DONDIA TORREYANA (S. WATS.) STANDL.) AND DONDIA INTERMEDIA (S. WATS.) HELLER

Seepweed is an almost infallible indicator of alkali. Occasionally land is found that is covered with it but is not saline, in which case the growth is weedy, coming in on abandoned land where the original vegetation had been destroyed. Land covered with a pure growth of it is strongly saline. Even where there are other plants and only scattered plants of the seepweed, the soil is usually quite strongly saline. If covered with a rank growth of the seepweed the water table is sure to be high. Seepweed land requires considerable leaching and often draining before it can be used profitably for farming.

MESQUITE AND CHAMISO (*PROSOPIS GLANDULOSA* TORR. AND *ATRIPLEX CANESCENS*  
(PURSH) NUTT.)

This land consists of sand dunes, in the Coachella Valley, and low sandy ridges, in the Gila Valley. In the former valley the total acreage of the sand dune area is large, but in the latter, where mesquite and chamiso are supplanted by chamiso the areas are small and scattered. In either case the land is very sandy and nonsaline. In the Coachella Valley the more level tracts of this land are being used for date and vegetable growing. These are relatively small areas, for the greater part of this type of land, the high sand dunes, can not be farmed. In the Gila Valley vegetables and grain are grown on this land.

SALTBUSH AND ARROWWEED (*ATRIPLEX LENTIFORMIS* (TORR.) S. WATS. AND  
*PLUCHEA SERICEA* NUTT.) COVILLE

Because of the high salt content and the high water table it is necessary to drain this type of land before it can be farmed permanently. In the Coachella Valley this type of land ought to drain readily because of the sandy layers in the subsoil while in the Gila Valley drainage may be more difficult since the subsoil is very heavy. Most of the land once farmed and later abandoned because of the high water table becomes overgrown with saltbush or saltbush and arrowweed. With proper drainage, however, good crops of alfalfa ought to grow again on this land.

PICKLEWEED (*ALLENROLFEA OCCIDENTALIS* (S. WATS.) KUNTZE)

This is the most saline of any of the types of land considered. Moreover the water table is high, so high that in spring the land is boggy and therefore drainage is essential if crops are to be grown.

SALTGRASS (*DISTICHLIS SPICATA* (L.) GREENE)

Meadows of saltgrass furnish a natural pasturage but the water table is so high and the salt content so great that drainage is necessary before crops can be grown on this type of land.

AGRICULTURAL POTENTIALITY OF THE LAND CHARACTERIZED  
BY EACH PLANT COMMUNITY

Probably the best type of land for irrigation agriculture is that characterized by a good stand of desert-sage. A good stand of creosote bush indicates land which has good drainage and which is free from alkali, but the soil is usually more sandy and not as productive as that occupied by the desert-sage. Mesquite and chamiso, and chamiso alone, indicate a very sandy soil free from alkali. Yucca and cactus lands, and giant cactus and paloverde lands, are too stony or the slope is too steep. Seepweed, saltbush, saltbush and arrowweed, pickleweed, and saltgrass lands must be leached and usually drained before they will be productive under irrigation. The adaptability of lands, characterized by different types of vegetation, for crop production under irrigation is shown in Table XXXV.



TABLE XXXV.—*The capability of lands for crop production as indicated by the principal plant communities of the Southwestern Desert*

Type of vegetation	Condition of vegetation	Is land capable of crop production under irrigation?
Creosote bush-----	Good growth----	Yes.
	Poor growth----	Doubtful because of stony soil or layer of rock or hardpan.
Desert-sage-----	Good growth----	Yes.
	Poor growth----	Doubtful because of presence of hardpan or alkali or both.
Mesquite and chamiso-----	-----	Not the greater part, small more level tracts for special crops.
Chamiso-----	-----	Yes.
Mesquite thicket-----	-----	Yes; if handled so that salts are leached out.
Seepweed-----	Rank growth----	Probably if secondary growth on abandoned land. If virgin growth, salt must be leached out and land drained.
	Poor growth----	Salts must be leached out. Drainage may be necessary.
Saltbush and arrowweed.	-----	Yes; if it is drained.
Saltbush-----	-----	Do.
Pickleweed-----	-----	Only if it is drained and salts are leached out.
Saltgrass-----	-----	Do.
Yucca—cactus and giant cactus—palo-verde	-----	Usually too stony or slope too steep.

## PLATE 1

A.—General view of the bad lands northwest of Indio, Calif. The vegetation, largely annuals, is very sparse. Indio, Calif., Apr. 20, 1913.

B.—Yucca and cactus association. Detail showing the rough and stony character of the land usually occupied by this association, and also the variety of plant forms including *Ferocactus acanthodes* (Lemaire.) Britton & Rose, *Opuntia bigelovii* Engelm., and *Encelia farinosa* A. Gray. Near Palm Canyon, Calif., March 8, 1915.





## PLATE 2

A.—*Parosela arborescens* (Torr.) Heller characteristic of washes in this portion of the desert. Many other plants are usually associated with it. West of Imperial, Calif., April 21, 1918.

B.—Giant cactus and paloverde association. Illustrates the rough and stony character of the soil and the variety of plant forms. Prominent in the picture are *Covillea glutinosa* (Engelm.) Rybd., *Franseria dumosa* A. Gray, *F. deltoides* Torr., *Carnegiea gigantea* (Engelm.) Britton & Rose, *Ferocactus wislizeni* (Engelm.) Britton & Rose, *Opuntia bigelovii* Engelm., *Fouquieria splendens* Engelm., *Cercidium torreyanum* (S. Wats.) Sarg., *Encelia farinosa* A. Gray, etc. North of Sacaton, Ariz., March 13, 1915.

### PLATE 3

A.—Giant cactus and paloverde association. A more level area showing *Carnegiea gigantea* (Engelm.) Britton & Rose, *Cercidium torreyanum* (S. Wats.) Sarg., *Olneya tesota* A. Gray, *Opuntia bigelovii* Engelm., *Covillea glutinosa* (Engelm.) Rydb., *Franseria dumosa* A. Gray, etc. Near Sacaton, Ariz., March 13, 1915.

B.—Creosote bush association. A pure stand of *Covillea glutinosa* (Engelm.) Rydb., with wide spaces of bare soil between the plants and, in the background, a very few associated shrubs. Nipton, Calif., September 14, 1915.







#### PLATE 4

A.—Creosote bush association. A pure stand of *Covillea glutinosa* (Engelm.) Rydb. The wide spaces between the plants are well covered with an ephemeral growth of *Plantago erecta* Morris. This condition is found only following a period of rainy weather, and the annual vegetation entirely disappears during the long drought periods. (See Pl. 3, B.) Chandler, Ariz., March 15, 1915.

B.—A plant of *Covillea glutinosa* (Engelm.) Rydb. showing the loose open branching of the top and the spreading habit of the root system which is well adapted to utilize the soil moisture of the wide spaces between the plants. Indio, Calif., March 7, 1915.

## PLATE 5

A.—Creosote bush and bur-sage. An open stand of good *Covillea glutinosa* (Engelm.) Rydb. with many plants of *Franseria dumosa* A. Gray occurring in the interspaces. The *Covillea* with its dark green lacquered leaves appears almost black, and contrasts sharply with *Franseria* which is silvery in color and, at a distance, appears almost pure white. Palm Springs, Calif., March 7, 1915.

B.—An open stand of creosote bush with desert-sage. The contrast in color of the two plants is very sharp. *Covillea glutinosa* (Engelm.) Rydb. is very dark green and *Atriplex polycarpa* (Torr.) S. Wats. an ashy gray. Death Valley Junction, Calif., September 15, 1915.





#### PLATE 6

A.—In the foreground *Isocoma* has come in to occupy the land previously covered with desert-sage. The original growth of desert-sage is shown in the middle of the photograph with thickets of mesquite in the background. The mesquite is here parasitized by *Phoradendron californicum* Nutt. Indio, Calif., March 21, 1913.

B.—Desert-sage association. A pure stand of *Atriplex polycarpa* (Torr.) S. Wats. presenting a uniform, ash-colored, shrubby growth about 3 feet in height. Such vegetation characterizes the best agricultural land of the desert valleys. South of Indio, Calif., March 6, 1915.

#### PLATE 7

A.—A single plant of seepweed showing the nature of the top and the root system to a depth of 2 feet. A salt crust which covers much of the seepweed land is here clearly shown. Thermal, Calif., March 5, 1915.

B.—A plant of *Atriplex polycarpa* (Torr.) S. Wats. showing the character of the root system with a tap root of over 5 feet. At a depth of 1 foot many laterals are developed, indicating that the plant receives most of its growth water from about this depth of soil. Near Indio, Calif., March 7, 1915.

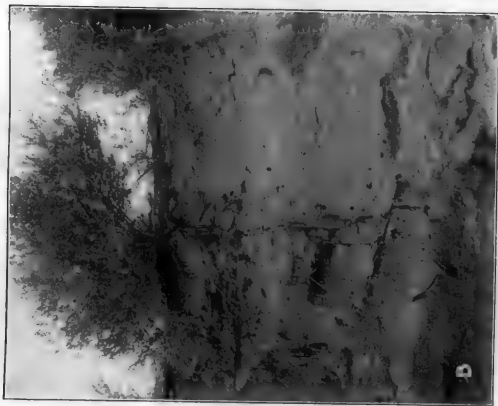






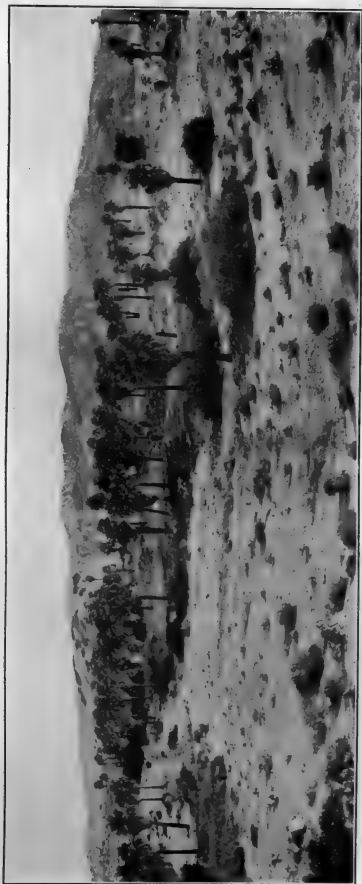
PLATE 8

A.—Saltgrass association. An even stand of *Distichlis spicata* (L.) Greene with *Allenrolfea* in the background. This photograph shows boring No. 12, Table XVII. Thermal, Calif., September 14, 1914.

B.—Pickleweed association. Practically pure *Allenrolfea* with bare salt incrustated areas between the plants. The plants are dark brownish green during much of the season. Sample 9, Table XX, was taken at this place. Thermal, Calif., September 14, 1914.

PLATE 9

Washington palms. A scattered growth of *Washingtonia filifera* Wendl. at the upper edge of the valley just below the clay hills showing the associated *Juncus robustus* S. Wats., *Imperata hookerii* Rupr., *Distichlis spicata* (L.) Greene, etc. North of Indio, Calif., April 20, 1913.





## PLATE 10

A.—A single plant of *Allenrolfea* inverted on the salt crust to show the character of the root system. The root extended to a depth of 2 feet. Thermal, Calif., March 6, 1915.

B.—Mesquite and chamiso community. Only the upper branches of the mesquite stand above the sand. The lighter-colored plants contrasting with the darker foliage of the mesquite are chamiso bushes. Las Vegas, Nev., September 11, 1914.

PLATE 11

A.—A pure stand of chamiso with many annuals occupying the interspaces as it appears on the "sand bars" in the Gila Valley. This photograph was taken at boring No. 2. (See Table XXII.) Chandler, Ariz., March 16, 1915.

B.—An old plant of *Atriplex canescens* (Pursh) Nutt. (chamiso) illustrating the habit of its roots on a sandy soil. The lateral root measured more than 9 feet. The top, with very few leaves, is characteristic of the plants at this time of the year. Indio, Calif., September 15, 1914.







**PLATE 12**

**A.—A saltbush, showing the large size which it attains under favorable conditions. Mecca, Calif., September 16, 1914**

**B.—A rank dense growth of arrowweed in Coachella Valley. Photographed near sample 15. (See Table I.) Indio, Calif., September 15, 1914.**

PLATE 13

A.—Seepweed land illustrating an area where “seep” or water heavily charged with salt has come to the surface and incrusts an old plant of seepweed. Thermal, Calif., March 5, 1915.

B.—Revegetation on seepweed land, a dense growth of young seepweed plants following breaking, and, in the background, sand dunes covered with mesquite. Indio, Calif., March 7, 1915.

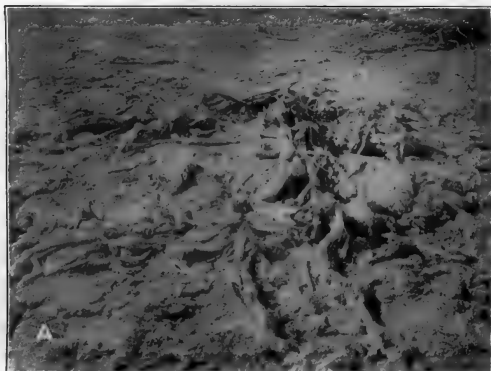




PLATE 14

A.—Seepweed association. A dense stand of seepweed with the soil area between covered with salt incrustations. This photograph was taken near sample No. 13. (See Table XXVI.) Thermal, Calif., September 14, 1914.

B.—A bare flat, slick during wet weather, but hard as cement during the dry season. No plants occur on such areas. Mina, Nev., September 29, 1916.

# ACROMANIA, OR "CRAZY-TOP," A GROWTH DISORDER OF COTTON<sup>1</sup>

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## THE NATURE OF GROWTH DISORDERS

Some of the diseases of cotton are manifested by abnormal growth of the plants, without localized injuries like those associated with parasitic fungi or bacteria. A remarkable feature of growth disorders is that the plant characters and habits of growth may be changed suddenly, the normal characters being replaced by abnormalities of many kinds and degrees. The changes of characters often are so complete that the abnormal growth would not be recognized as belonging to the same variety as the early growth of the same plant, or even to the same species, if the connection were not known. To appreciate the extent of the abnormalities, it is necessary to be familiar with the normal characters and development of the plants, as in the study of breeding and adaptation of varieties.

Similar disorders affect other plants, and some of them are known as mosaic diseases, because the leaves are discolored in irregular patchwork areas of light and dark green. But in crazy-top the growth is abnormal in other ways, without discoloration of the leaves. Some of the disorders are temporary, with a later return to normal growth, while other disorders are permanent so that the abnormalities continue to appear in all of the new growth that takes place. When the changes of characters are permanent the growth disorders appear somewhat analogous to "bud mutations."

Since the symptoms of crazy-top are aggravated by stress conditions, it might be expected that the cause would be found in some peculiarity of the environment. The presence of harmful substances in the soil is easy to assume and difficult to disprove, but with crazy-top there is no indication that any particular type or condition of soil is associated. The disease occurs under a wide range of cultural conditions, and appears sporadically in scattered individuals, as well as in more compact groups of plants, or in large areas. The mode of occurrence of crazy-top is consistent with the possibility of an infection distributed by insects. It is known that several of the mosaic diseases of other plants are carried by insects, and that some of the diseases are very infectious, so that only slight surface contacts are necessary to spread the "virus."

The underlying cause of mosaic diseases is still obscure, even with those that have been studied most carefully, as in sugar-cane, sugar-beets, tobacco, and potatoes. Some pathologists credit the idea of very minute Protozoa, smaller than bacteria, beyond the limit of microscopic vision, as indicated by the passage of contagious "virus" of mosaic disorders through filters that are impermeable to bacteria. Other investigators have sought for causes of such diseases in organic poisons, or in the reactions of organic ferments or "enzymes." The causes, no doubt, are different for the disorders that are not permanent nor infectious, though one of the temporary disorders of cotton is due to plant lice. Another may be a physical injury or traumatism, primarily affecting the oil-

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<sup>1</sup> Received for publication May 1, 1924.

glands. Still another class of disorders might be described as genetic, since they either are inherited or are closely connected with genetic factors.

If organisms are involved in crazy-top, they doubtless are able to live continuously in the cells without destroying the protoplasm, like the bacteria that produce crown-galls. Organisms that cause permanent disorders must be adapted or adjusted in some manner against increasing to large numbers that would destroy the host-plant. Since it would be to the advantage of such parasites to injure their host-plants as little as possible, the tendencies of natural selection with parasites as well as with the plants themselves would be in the direction of less serious injuries. Thus a condition of immunity might be reached in which little or no injury would be apparent. An immunity of tolerance might exist in some species of plants and an immunity of resistance in others.

The different degrees to which the adjoining areas of leaf tissue are affected, as shown in the mottled discolorations of the leaves in the mosaic diseases, would indicate delicately balanced conditions of the tissues in relation to the disease. It is easy to understand that an organism or a chemical compound thus limited in its existence to the vegetative protoplasm of the plant might be very difficult to isolate. That growth disorders are not conveyed through the seeds may mean that the cause of the disease is too delicate or unstable to survive a resting stage. One of the growth disorders of cotton is limited to cool weather in the spring and fall. Malformations induced by chemical substances may be seen in the formation of galls through secretions or excretions of insects, mites, or nematodes. The growth disorders might be considered as slight, generalized gall-formations.

Investigators of animal nutrition now recognize special growth-controlling substances called vitamins and hormones whose absence or deficiency renders development abnormal, and results in such diseases of children as rickets, acromegaly, or cretinism. Some of the vitamins that are necessary for animals are derived from plants and may have their proper functions in the growth of the plants, so that plant disorders might result if the vitamins were destroyed or counteracted by other substances. Thus an anti-vitamin substance or a physiological disturbance that destroyed or disturbed the vitamin relations might be the cause of a growth disorder of a plant.

On account of the nature of the plant organization, growth is not restricted to one period, but is continued or renewed at intervals, so that a growth disorder is continued or repeated through the life of the plant individual. Thus it is possible to understand that such disorders as crazy-top may be similar to some of the diseases of children or of young animals. But until the causes of the plant disorders are more definitely known, investigation must be directed largely to the symptoms, as manifested in the behavior of the plants. Hence, a comparison of crazy-top with other growth disorders apparently is the best way to facilitate the further study that is needed.

#### GENERAL FEATURES OF CRAZY-TOP

Reference was made in the Journal of Heredity for October, 1923, to the crazy-top disorder in Arizona as possibly analogous to other growth disorders of cotton that have been studied in China and in Haiti. Further observations in Arizona showed additional features in common with the foreign disorders. As indicated by the popular name, the plants infected with crazy-top undergo a sudden change in their habits of growth, the upper growth of affected plants becoming abnormal and strikingly different from the earlier normal growth of the same plants. (See Pl. 2.)

The name *acromania* is suggested as a technical equivalent of crazy-top, conveying the same idea of abnormal behavior of the upper part of the plant.

Although the differences between the normal growth and the crazy-top growth are usually less striking than in the disorders in foreign countries, in other respects there are close analogies. After a period of normal development early in the season, the plant characters are changed abruptly and all of the later growth not only is abnormal, but shows many kinds and degrees of abnormality. The diversity of the affected plants renders it more difficult, of course, to recognize the disease, especially in scattered individuals, but when the crazy-top plants occur in large groups or areas there seldom is any difficulty in distinguishing the normal from the abnormal individuals.

One of the most general and striking features of crazy-top in Pima cotton in Arizona is the change in the form of the branches at the top of the plant, which suggested the name of the disorder. The upper branches of the affected plants usually are stronger and more upright than the normal horizontal fruiting branches developed from the lower joints of the same stalk (Pl. 2). The abnormality may be considered as a suppression of fruiting branches, or as a partial transformation of fruiting branches into vegetative branches. The transformation usually is not complete, as shown by the presence of a small floral bud or bud-scar at each joint of an abnormal branch. Some of the plants abort their buds at very early stages, as indicated by very small bud-scars, while other plants develop their buds nearly to the flowering stage before shedding. The abnormal branches also show frequent examples of fasciation or adhesion, where the branches divide but the bark remains united.

The sterility of the crazy-top plants is the feature of most interest to the farmer. Not only the abnormal upper branches are sterile, but usually the whole plant. (See Pl. 2.) A general shedding of buds and young bolls is considered by S. H. Hastings as the first symptom of an outbreak of the disease. This would explain why the normally formed lower fruiting branches are so generally sterile on the crazy-top plants, though sometimes a few bolls are retained. The extent of shedding may depend upon the conditions at the time of infection, or the injury might not become apparent until stress conditions were encountered. Of course, the symptoms are more striking and the losses greater when the crazy-top plants are in large groups or areas.

If there are no bolls to open, the crazy-top areas show notable contrasts with the other portions of a field at the end of the season. (See Pl. 1.) Such contrasts are more striking in Upland cotton than in Pima because the Upland bolls are larger and the fiber is pure white, while the Pima bolls are smaller and more evenly distributed, and the fiber has a buff tinge. Also the affected plants of Pima are not so completely sterile, but usually produce at least a few bolls at the top of the plant late in the season. With favorable cultural conditions a few of the affected plants of Upland cotton may set bolls late in the season, though mostly very small and imperfect.

### CRAZY-TOP MORE SEVERE IN UPLAND COTTON

In former years, when the Pima cotton was grown exclusively in the Salt River Valley, the crazy-top abnormality was considered by some as an indication that the variety was "running out," and even urged as a reason for planting Upland varieties. But with Upland cotton planted extensively in 1923, and crazy-top appearing in many of the fields, that explanation of crazy-top could no longer be maintained, although many of the growers did not recognize the disease in Upland cotton on account of the different symptoms.<sup>2</sup>

<sup>2</sup> In this connection it may be noted that the average yield per acre of Pima cotton in 1923 was the highest since the variety began to be grown commercially. Also a careful comparison of old and new seed stocks in 1922 gave no evidence to support the idea of running out. See Kearney, T. H. (12).<sup>3</sup>

<sup>3</sup> Reference is made by number (italic) to "Literature cited," p. 827.



In some of the Upland fields the behavior of the affected plants is much more abnormal than with Pima. The size of the plants is notably restricted by the shortening of the internodes, and the leaves are dwarfed and distorted, as shown in Plates 10 and 11. Such deformities of the leaves apparently do not occur in Pima cotton, but are quite similar to the effects of the stenosis disease on Upland cotton in Haiti. That the same disorder might produce different symptoms, or that differences of susceptibility might be shown in the various types of cotton, had also been learned from the previous study of the cotton disorders in Haiti and in China. Hence it was possible to suppose that the same disease could be responsible for the abnormalities of the Upland varieties, as well as of Pima.

The leaf injuries of Upland cotton in China were less than those of Upland cotton in Haiti, but in both cases the Upland cotton suffered worse than the Sea Island. Pima cotton in China showed relatively little of the mosaic discoloration, and the leaves were not crumpled nor the internodes shortened as in the native Chinese varieties. But the Pima cotton in China lost most of its leaves from black-arm, as though the susceptibility to that bacterial disease had been increased by the growth disorder. The cotton that suffered worst from the growth disorder in China was the native Asiatic type, although an Indian variety of the Asiatic type with hairy leaves and stems appeared to be quite immune to the cyrtosis disorder (Pl. 8). Similar differences of susceptibility to growth disorders or mosaic diseases are known in other plants. Although the crazy-top disorder does not show the usual symptoms of a mosaic disease, since there is no definite discoloration of the leaves and relatively little distortion, other features are closely parallel.

#### OTHER GROWTH DISORDERS OF COTTON TO BE DISTINGUISHED FROM CRAZY-TOP

Since the present interpretation of crazy-top would not have been reached without a previous study of other growth disorders, a review of such information seems necessary to give a clear understanding of the distinctive features of crazy-top. In addition to the disorders studied in China and in Haiti, to which reference has been made, the symptoms of crazy-top may be confused with other disorders which are of common occurrence in the United States, as brachysm, tomosis, and hybosis. Two or more disorders often appear concurrently in the same plant, and to some extent one disorder may be induced or intensified by another, so that the symptoms are variously combined.

These maladies are described as disorders, rather than as diseases, because they are not of the same nature as the better known parasitic diseases of plants, caused by fungi or bacteria. Some of the disorders appear to be of the same nature as the so-called mosaic diseases of other plants which in some cases are known to be communicated by insects. Patch-work mottling of the leaf tissues with areas of darker and lighter green is a prominent feature in the cotton disorder of China, but does not appear in crazy-top.

#### BRACHYSM, OR CLUSTERING

The name brachysm has been applied to an abnormal shortening of the internodes of the fruiting branches of frequent occurrence in Upland cotton, and usually accompanied by malformations of the sockets where the pedicels of the bolls are inserted on the internodes of the branches. Instead of a separable joint between the pedicel and the internode there is a more or less complete fusion. Hence in brachytic varieties the abortive buds or young bolls are not shed, but remain attached to the plant, even after they become shriveled and

dry, so that brachytic plants are sometimes considered as diseased on this account (5).

As a morphological change, brachysm represents an acceleration or stepping forward in the course of development toward the fruiting stage, so that the normal differences between the internodes and the pedicels are reduced. It may be considered that the internode-pedicels of brachytic plants are formed by a process of metamer-hybridism, combining the characters of two successive structures that normally are distinct. In other words, there is a breaking down of the normal differentiation of the parts, or intermediate expression of the characters that are normally distinct, a type of variation to which the term metaphanic has been applied.

Other metaphanic variations may be seen in the leaves of brachytic internodes which often are reduced and partially transformed into involucre bracts. Also the formation of the bracts is often abnormal in brachytic varieties, some bracts being more leaf-like, while others may be reduced and variously united with the calyx.

Though brachysm is considered as a normal character of some of the Upland varieties, and such "cluster-cottons" may be very productive under favorable conditions, the brachytic habit is considered undesirable and to be avoided in breeding. The brachytic varieties generally are very unstable and the tendency to brachysm may be so intensified that many of the plants are completely sterile when cultural conditions are unfavorable. Thus brachysm reaches a destructive stage when the abortion of flower buds is increased, or is carried to the point of total abortion, resulting in complete sterility. This may occur in only a few individuals, or the sterile plants may be so numerous that the yield is greatly reduced.

Various degrees of the brachytic tendency are shown. Some varieties that are not usually brachytic may have, under some conditions, very short fruiting branches. From the same stock of seed many brachytic plants may appear in some places, while in other places no indications of brachysm may be shown. In the Durango variety brachytic plants occur frequently in some of the irrigated valleys of Arizona and California, but may be quite absent in fields grown in South Carolina or in southern Virginia from the same stocks of seed, and similar behavior in relation to brachysm is indicated in the Acala variety. Even on the same plant, it often happens that some of the fruiting branches are normal while others are distinctly brachytic. Such differences of behavior have not been explained, but in view of the fact now recognized, that branching habits may be changed by growth disorders, it seems possible that some occurrences of brachysm may be connected with crazy-top or other growth disorders. Some features of crazy-top are similar to brachysm, or to abnormalities of branching that are to be associated with brachysm (Pl. 4). The late-season fruiting branches of Pima cotton have the internodes very unequal, and the sockets of the pedicels defective, much as in cluster varieties of Upland cotton. (See Pl. 3.)

#### TOMOSIS, OR LEAF-CUT

The chief feature in tomosis is the mutilation of the tissue of the leaf, with irregular perforations or marginal erosions, accompanied by more or less crumpling or other distortion (Pl. 5). The mutilations result from the death of portions of the leaf-tissue at early stages of growth. The dead areas are more frequent along the folds midway between the principal veins or along the margins, but may appear in any part of the leaf, and often with partial healing or regenera-

tion of the edges of the wounds. Adhesions may be formed in the scars and are the cause of some of the distortions of the mutilated leaves.<sup>4</sup>

Tomosis is of general occurrence in all of the cotton-growing districts of the United States, as well as in foreign countries. Though extensive tomosis injuries are usually confined to the seedling stage of the plants, a few injured leaves are usually to be found at later stages of growth. Occasional individuals show many tomosis mutilations through the season, and such plants are likely to be sterile or abnormal in other ways. Similar cases of congenital tomosis are likely to be found in hybrid stocks as one of the abnormalities that appear in the second and later generations. Also it appears that tomosis is more frequent in plants that are affected with other growth disorders than in normal plants under the same conditions.

The death of the leaf-tissue in tomosis has been traced to the oil-glands, which apparently are killed in advance of the neighboring cells. Under the microscope it is possible to see that the cells around the oil-glands are injured before they are killed. The chloroplasts become discolored and then disintegrate and disappear, leaving the cells transparent. Along the edges of the wounds some of the injured cells survive, but with no green color, the loss of the chloroplasts being permanent (Pl. 5, B, C, D). The injury to the chloroplasts suggests an analogy with the mosaic diseases, though the course of the disease is very different. The tomosis injuries are much more acute but more definitely limited, and with no subsequent weakness around the injured areas of leaf-tissue.

Though tomosis is very frequently accompanied by another disorder called hybosis, or leaf-curl caused by plant lice, which also affects cotton in the seedling stage, tomosis injuries may be severe where there are no plant lice, and no relation to other insects has been found. That the occurrence of tomosis is so general in itself is a reason for supposing that the injuries are not due to a parasite but are a general effect of some unfavorable condition, such as the wider and more sudden changes of temperature to which the young plants are exposed during the early stages of growth.

When tomosis is severe the growth of the seedling is retarded, and the terminal bud may be aborted, so that the plants are crippled and deformed, and generally produce fewer bolls than their normal neighbors. Except in these cases the effects of tomosis appear to be strictly temporary. Though all of the plants in a field may be badly affected, with the early leaves badly mutilated and deformed, such a period of distress may be followed by a general and rapid recovery with all of the new growth quite normal, as soon as the weather is favorable. (See Pl. 5, A.)

If the plants are not thinned too early the injured individuals are easily recognized and removed. Also there is less exposure to extreme conditions if the young plants are close together in the rows.

Tomosis occurs in all of the principal types of cotton, though some are more susceptible than others. In Arizona and California the Pima cotton usually is appreciably less susceptible to tomosis than the Upland varieties. Earlier recovery of Pima cotton from tomosis no doubt is connected with its ability to grow at lower temperatures. The Upland varieties remain dormant for a longer period while the weather is cold and also lose more time in the stage of susceptibility to tomosis.

With Pima and Sea Island varieties growing under the same conditions, at Sacaton, Ariz., the tomosis injuries of Sea Island cotton are more serious and

<sup>4</sup> COOK, O. F., LEAF-CUT OR TOMOSIS, A DISORDER OF COTTON SEEDLINGS (4). See also Reports of the Chief of the Bureau of Plant Industry, U. S. Department of Agriculture (10, 11, 13, 16). Cook, O. F., Results of cotton experiments in 1911 (2).

continue much later in the season, so that the plants may not recover in time to mature a full crop. The deformity and mutilation of the leaves were still severe in the Sea Island cotton after adjoining rows of Pima cotton had entirely recovered. Upland cottons also suffer severely from tomosis, and may be outgrown and outyielded by the Egyptian cotton, under conditions in the Southwest.

#### HYBOSIS, OR LEAF-CURL

The chief symptoms of hybosis are reduction and distortion of the leaves, but without the perforations or mutilations which are characteristic of tomosis. The two disorders are commonly found together on the same plant or on the same leaf, but may also occur separately (4).

The hybosis distortion is an upward swelling or crumpling of the tissue of the leaves, and usually is greater between the principal veins near the base of the leaf. The plant lice producing the injuries shown in Plate 6 were identified as *Aphis gossypii* Glover, a species that is widely distributed. Plant lice are regularly present with hybosis, and apparently are the cause of this disorder, though the extent of the malformations of the leaves depends upon the temperature as well as upon the presence of the insects.

The greatest malformations occur during the cool weather of the spring months, when the cotton is making only a slow growth (Pl. 6). As soon as the weather is warm enough for the cotton to grow rapidly the growth becomes normal. The altered behavior of the plants may be ascribed to the warmer weather, since the plant lice may still be present, though usually they become less numerous in midseason, and increase again in the fall. Also the hybosis symptoms return as the weather becomes cooler.

In some cases the recovery from hybosis is not uniform, and the growth of the plants in definitely restricted spots or areas may continue to show severe hybosis injuries while over the rest of the field the plants are producing normal leaves. Such irregularities were noted in fields of Pima cotton in the Salt River Valley, June 28, 1920, and a photograph secured which shows the extent of the hybosis deformity, continued later in the season than usual. No reason was found for the striking difference of behavior. The suggestion of alkali spots was considered, but seemed improbable. The possibility that such plants had been infected with crazy-top or some other disorder, which could intensify the hybosis effect, may be worthy of consideration.

The appearance of late-season hybosis in Mebane cotton at Sacaton, Ariz., in November, 1923, is shown in Plate 7. The internodes are much shortened and the leaves crumpled, though the size of the leaves and of the involucre bracts is not much reduced, and there are gradual changes from the normal to the distorted condition. Thus it is easy to distinguish the late-season hybosis from crazy-top, although from a distance the two might be confused. Hybosis is likely to be accompanied by tomosis, which is true also of crazy-top. The lacerated leaf at the lower right-hand corner of Plate 7 may be an example of tomosis, or possibly of insect injury. The plant lice were quite numerous on the specimen photographed, and of a very dark greenish color.

Hybosis is a prominent feature in the black-land prairie region of Texas, which is now the chief center of cotton production in the United States. The surface soil of the black-land prairies is likely to be wet and cold in the spring, and the growth of the cotton may be restricted for several weeks by a heavy infestation of plant lice, and the resulting distortion of the leaves may be very severe, though without a permanent injury to the crop. On the contrary, many farmers believe that it is good for the cotton to become "lousy" in the early stages. The restriction of growth early in the season is really an advantage under the black-land

conditions, since a larger growth of the plants would result in more serious checking by dry weather later in the season. Cotton that is planted on more open bottom-land soils often grows much faster and begins flowering much earlier, but is not so likely to mature a good crop as the cotton that grows on the heavy prairie soil.

According to reports from South Africa, serious injuries to cotton may occur from insects of the family Jassidae, with distortion and discoloration of the leaves, but later recovery of normal habits of growth in the dry season, when the insects disappear. Thus the jassid disorder in Africa seems most analogous to hybosis. Varieties of cotton with hairy leaves and stems are considered immune to the jassid disorder (18).

#### CYRTOSIS, OR CLUB-LEAF

This disorder is most severe in the Asiatic type of cotton, and is a limiting factor of cotton production over a wide region in the central part of China (Pl. 8). The disease apparently is of general and regular occurrence, with the late growth of all the plants affected. The development usually is normal to the flowering or early fruiting stage, with later abrupt transitions to the club-leaf condition. The leaves of the abnormal growth are reduced, distorted, and discolored, with mottling of dark and light green, especially along the margins and between the principal veins. The internodes and petioles are shortened, and small super-numerary branches are frequent, so that the abnormal growth may form compact masses of foliage, especially in the native Chinese cotton.<sup>5</sup>

When the disorder is severe all of the floral buds may be aborted, so that the crop is restricted to the early bolls. Cotton that is planted too late, so that bolls are not set before the cyrtosis begins, may remain completely sterile, especially under conditions of exposure to dry hot weather, which intensifies the cyrtosis symptoms. Thus the cotton suffers less from cyrtosis in the humid coast districts around Shanghai and Nantung than farther up the Yangtse Valley, at Nanking, Nanchang and Wuchang. In the more northern districts, from Hankow to Peking, there is less injury to the crop, on account of a later incidence of the disease.

In Upland and Sea Island cotton grown in China, cyrtosis does not shorten the internodes or reduce and curl the leaves to the same extent as in Asiatic cotton, so that the aspect of the affected plants is quite different. In Upland cotton the distorted leaves have the margins curled under (see Pl. 8, E), while in Sea Island cotton the margins are tilted up, so that the upper surface of the leaf becomes more deeply concave or channeled.

The occurrence in India of a disease that is closely similar to the cyrtosis disorder, if not the same, is shown in a paper by Kottur and Patal (14). It appears, however, from this account that the disease in India is of irregular occurrence, instead of being practically universal as in the cotton districts of China. This may indicate that most of the Indian cotton is immune to cyrtosis, or that the agents or sources of infection are less common than in China. The writers ascribe the disease to adverse conditions of growth, and state that it occurs much more extensively in some seasons than in others.

#### STENOSIS, OR SMALLING

The striking feature of stenosis is the extreme reduction in size that may take place in the internodes, leaves, and floral organs of the affected plants, in addition to deformity and discoloration of the leaves, as in the cyrtosis disease of

<sup>5</sup> COOK, O. F., A DISORDER OF COTTON PLANTS IN CHINA (6). See also Report of The Chief of the Bureau of Plant Industry for 1921, p. 23 (17).

China. The smalling disease was seen in July, 1923, in the vicinity of St. Michel, in the north-central part of Haiti. In some of the fields all of the plants appeared to be affected, while in other fields only scattered individuals showed the disease while the intervening plants had continued to grow normally through the season. In some cases it appeared that plants had become affected at different stages of growth, as indicated by the extent of the top-growth that had occurred after the incidence of the disease (8).

On account of the reducing effect of this disease in Upland cotton, the contrasts between the normal and abnormal growth are most striking. Also, there may be a very wide range of diversity in the abnormal growth of different individual plants in the same field of cotton, not only in the extent of dwarfing but in many other features of the abnormal growth, including the ability to produce flowers or bolls. The abnormal growth of many of the plants is completely sterile, either by suppression of flower buds or by abortion of the buds at very early stages. In other plants the flower buds may develop to larger sizes, or the flowering stage may be reached. Some plants produce many undersized flowers but set no bolls, or on some of the less injured plants bolls may be retained and grow to nearly normal size.

In the examples of stenosis shown in Plate 9 the abnormal growth began very abruptly, with the leaves and other organs suddenly restricted to very small size, after producing normal leaves at the lower nodes of the same stalks. One of the plants had grown less than three inches in height after the disorder began, though small branches from several nodes along the stalk showed the same dwarfing and other abnormalities as the top-growth. The other plant was less seriously affected, and retained its squares to larger size. The zigzag form of the stalk in this specimen is one of the many peculiarities that contribute to the diversity of the abnormal top-growth of the affected plants.

No indications of recovery from stenosis were observed, even in fields where the disease had remained limited to scattered individual plants that must have been infected several weeks before, as shown by the amount of abnormal top-growth that had developed. The absence of more recent infections would seem to show that the insects or other agents of infection were no longer present in the fields. Later plantings of Upland cotton, in July and August, 1923, were reported in November to have shown no indication of being affected with the disease.

The mosaic discoloration of the leaves, though generally appreciable in stenosis, is a less prominent feature than in cyrtosis, while the reduction or dwarfing of leaves and other organs is carried much farther, especially in Upland cotton. On account of the extreme dwarfing of the new growth in some of the affected plants, the range of variation in the symptoms appeared much greater in the stenosis disease in Haiti than in the cyrtosis disease in China.

The Sea Island type of cotton was represented by occasional plants in some of the fields and appeared to be much less affected than the Upland cotton, as had been observed also in China. The Bourbon or "Native Haitian" cotton did not appear to be injured at all by stenosis, though it may be tolerant of the disease, rather than immune. That the disease may be carried in the Bourbon cotton is indicated by a statement of G. G. Burlingame in April, 1924. A later planting of Upland cotton at St. Michel, Haiti, adjacent to a block of Bourbon cotton, showed a large proportion of diseased plants in the rows next to the Bourbon cotton, and gradual reductions in the number of diseased plants in the rows farther away.

## DIAGNOSIS OF ACROMANIA, OR CRAZY-TOP

In Pima cotton the distinctive features of crazy-top are the abnormal branching and sterility of the affected plants. In Upland varieties the symptoms are more pronounced, including reductions and distortions of the leaf-blades, petioles internodes, involucre bracts and floral organs, with all stages of sterility from the complete suppression of the floral buds to the development of small unsymmetrical bolls. Under extreme conditions the reduction and distortion effects in Upland cotton may equal those of the stenosis disease in Haiti, but in Arizona the symptoms meliorate as the season advances, and the diseased growth becomes less abnormal. Thus flowering and fruiting may be resumed by many of the Upland plants before the end of the season, and in Pima cotton the production of late-season bolls on the abnormal top-growth is a regular feature.

When the leaves are reduced in size the lobes may be partially suppressed. The leaves may be aborted in the upper part of the plant while the floral buds are developed, or the leaves may be retained and the floral buds suppressed. The leaves are not discolored as in the mosaic diseases, and there is less tendency to crumpling or other distortion. Branching is very irregular, with fruiting branches largely suppressed or transformed into vegetative branches which also are very irregular in size. The involucre bracts are variously reduced or divided, the flowers often very small and the anthers defective so that pollen may be completely lacking.

## ANALYTICAL SYNOPSIS OF GROWTH DISORDERS OF COTTON

Although crazy-top apparently is more analogous to cyrtosis and stenosis, the other disorders, brachysm, tomosis, and hybosis, are present in Arizona and need to be distinguished in the study of crazy-top. Hence the following synopsis is given in the form of an analytical key to assist in the study of the growth disorders and emphasize the contrasting characters:

Leaves not distorted, discolored, or reduced in size; malformations restricted to the fruiting branches, which have short joints more or less fused with the pedicels, so that aborted buds or bolls remain attached to the plant... *Brachysm*.

Leaves distorted or discolored or the plants abnormal in other ways.....

Leaves with irregular perforations or eroded margins, resulting from death of portions of the leaf-tissue, beginning at the oil glands..... *Tomosis*.

Leaves not perforated or mutilated, except as tomosis may accompany other disorders.....

Leaves deeply crumpled or bullate by inflation of the tissue between the veins, resulting from the presence of plant lice in cool weather..... *Hybosis*.

Leaves not regularly bullate and the disease not restricted to plant lice or to cool weather, but becoming more severe with higher temperatures.....

Top growth abnormal, but the leaves not discolored..... *Acromania*.

Top growth abnormal and the leaves with mottled discoloration.....

Leaves of Upland cotton greatly reduced and distorted but not regularly or strongly discolored..... *Stenosis*.

Leaves of Upland cotton not greatly reduced but the margins notably discolored and strongly decurved or rolled under..... *Cyrtosis*.

It may facilitate the study of the growth disorders of plants to have general terms available as well as specific names for the various disorders. The word *dysphytis* might be used as a general term for growth disorders, with *dysphyte* to refer to a plant that is made abnormal by a growth disorder. No particular feature has been observed which is common to all of the growth disorders but



only the general feature that the growth of the plants is changed and becomes abnormal, yet this apparently is a sufficient reason why the different disorders should be associated at least for purposes of study.

### CRAZY-TOP SYMPTOMS COMPARED EFFECTS UPON THE SIZE OF THE PLANTS

The dwarfing effects upon the internodes, petioles, and leaf blades are less pronounced in crazy-top than in cyrtosis or stenosis, and the foliage retains a healthy green color to the end of the season. In Pima cotton the size of the plants is but little affected, except that the crazy-top plants often grow a few inches taller than the adjacent normal plants, as though sterility had given the affected plants a greater vegetative vigor. The appearance of the upper part of crazy-top plants is like some sterile hybrids or like the rank growth of "boll-weevil cotton" when all of the buds are aborted through weevil attack. The impression of greater vigor in the crazy-top growth no doubt is increased by the resemblance of the upright abnormal branches to the normal vegetative branches which are produced from the lower nodes of the main stalk on rank-growing, widely spaced plants.

In Upland cotton the effects of the disease may be much more pronounced, especially if the cultural conditions are not favorable for the plants or periods of stress are encountered. Near the center of the crazy-top spot, shown in Plate 1, some of the plants were quite stunted. This might result from earlier infestation of such plants when little of the normal growth had been formed, which no doubt would result in a greater restriction of later development.

### ABNORMAL BRANCHING

The loss of the normal dimorphism of the branches, and the sterility of the affected plants are the most general as well as the most prominent feature of crazy-top. In Pima cotton the sterile crazy-top branches that replace the normal fruiting branches in the upper part of the plant are thicker and straighter than the normal fruiting branches and have an erect or ascending position instead of horizontal. Thus it could be said that formation of fruiting branches is suppressed by crazy-top, at least temporarily, during the period of stress or hot weather conditions.

In normal plants the two types of branches, fruiting and vegetative, are distinct, with definite functions and positional relations. The axillary buds produce vegetative branches, while the normal fruiting branches develop from extra-axillary buds, which stand at the right or at the left of the axillary buds. The abnormal branches that replace the fruiting branches and give the crazy-top plants of Pima cotton their peculiar and characteristic appearance are of intermediate forms, as sometimes occur in abnormal variations, like the so-called "bull-stalks" of Sea Island cotton, or in degenerate hybrids. Plants with such abnormal branches, intermediate between the vegetative and fruiting type, are often completely sterile, as a result of abortion of all of the flower-buds, usually at very early stages. Abortion of young fruiting branches was observed at Bard, Calif., several years ago and was considered an effect of over-luxuriant growth of the plants (3).

If growth continues on the lower fruiting branches of affected plants, abnormalities appear, like those of the top-growth. In some cases vegetative shoots are developed from axillary buds of the fruiting branch. In other cases the terminal portion of the fruiting branch changes its behavior, assuming the appearance of a vegetative shoot, and taking a more upright position, if the



weight is not too great to be supported. Abnormal supernumerary branches may also develop from axillary buds, though not to the same extent as in the cyrtosis disorder.

Floral buds continue to be formed on most of the abnormal crazy-top branches of Pima cotton, but usually the buds are aborted at very early stages, as shown by a small scar at the side of the leaf-axil. In other cases the transformation to vegetative branches apparently is complete, when no bud-scars are found and the stipular rims do not encircle the joints as in fruiting branches.

In Upland cotton greater changes occur in the branching habits, as in other features, as a result of crazy-top. In a field of Hartsville cotton near Casa Grande, Ariz., observed by Robert D. Martin in September, 1923, the fruiting branches were reduced to shapeless rudiments, and only a few of the affected plants retained any of their squares to appreciable size. Most of the squares were shed at very early stages of growth, and there were practically no flowers in the affected areas. (See Pl. 10 to 15.)

The later growth of the same plants, in October and November, was less abnormal. The fruiting branches were formed on most of the plants, though with very short, irregular, and abnormal joints. In November many of the plants were retaining their squares to larger sizes, though some of the plants failed to produce any squares or shed all of them in the minute rudimentary stages. Most of the squares were still being shed before flowering, though some plants were flowering and a few were holding some of their bolls. Thus there were many stages of sterility of the abnormal branches.

In Pima cotton the recovery of normal habits of branching is more general. Under less extreme conditions in latter part of season a more normal formation of branches is resumed. The buds are held, and a profusion of blossoms may give the crazy-top areas an appearance of fertility, but too late to make good the losses that have occurred. Most of the plants begin to set bolls, though inferior in size and with fewer seeds than normal bolls.

#### FASCIATION OR ADHESION OF BRANCHES

Fasciation or adhesion of branches is of frequent occurrence in plants affected with crazy-top. Examples of twinning or dichotomous divisions of the branches are not uncommon. The lower joints of such branches are often adherent at the base, and sometimes for the entire length of the joint, or the joints of several branches may be united. Adhesions between the internodes of the fruiting branches and the pedicels of the bolls are also very frequent.

Short axillary branches bearing a single boll on a long pedicel are often produced in Pima cotton at the upper nodes of the main stalk. In the crazy-top plants the long pedicel of this axillary boll often is more or less adherent with the basal joint of the fruiting branch. In such cases the axillary boll is usually abortive and the supporting branch may be dead although the fruiting branch is alive.

Many of the less abnormal branches that are formed at the top of the Pima plants late in the season are more or less fasciated or adherent, forming a rather dense cluster of leaves, flowers, and bolls, in contrast with the uniform open growth of the normal plants. Such clusters of small late bolls are found also in Upland cotton, but much less frequently than in Pima.

Fasciations or adhesions of large abnormal branches with main stalks of the plants were observed in a Pima field near Phoenix, Ariz., and noted as an extreme form of this abnormal tendency. In some cases the branch was nearly equal to the upper part of the main stalk and retained a nearly upright position, thus giving the appearance of a dichotomous division of the stalk. That such abnormal

growths represent transformed fruiting branches, rather than vegetative branches, is further indicated by the fact that they often have very long basal joints which is a character of fruiting branches, in contrast with normal vegetative branches which have short joints at the base.

#### SHORT AND IRREGULAR INTERNODES

Irregularity in the lengths of the internodes of the branches is a general feature of the abnormal growth. Instead of the normal gradation in the lengths of the internodes, an abnormal branch of Pima cotton had joints of the following proportions, beginning at the base: 7 inches, 3 inches,  $\frac{3}{4}$  inch,  $2\frac{1}{2}$  inches, 1 inch,  $\frac{3}{4}$  inch. These irregularities were accompanied by twinning, adhesion, and irregular development of the secondary and tertiary branches.

In many cases the late growth, even from distinctly abnormal crazy-top branches of Pima cotton, shows a return to nearly normal forms of fruiting branches, with the basal joints longer and the others gradually shorter. At the same time, more normal fruiting branches are produced from the upper nodes of the main stalk. The production of more normal fruiting branches from the very abnormal "false vegetative" branches was noted as a further evidence of the general consistency of behavior in all parts of a crazy-top plant. While the habits of growth of the affected plants are distinctly abnormal the responses to differences of external conditions are as great or even greater than with normal growth.

The photograph of a late-season fruiting branch in Plate 3, illustrating a partial return to normal behavior in the late-season growth of Pima cotton, still shows very unequal joints and malformations of the sockets of the pedicels as in brachytic varieties of Upland cotton. The malformations of the sockets explain the failure to shed the abortive squares, as in "cluster" varieties of Upland cotton. That the dead and shriveled squares often remain on the affected plants has been noted by Dr. T. H. Kearney as one of the striking symptoms of crazy-top in Pima cotton.

The shortening of the fruiting branches was carried much farther in the Hartsville variety than in Pima, often to the extent of complete suppression or reduction of the branches to shapeless rudiments, less than an inch long (see Pl. 11 and 12). Even the more normal fruiting branches of Hartsville, formed late in the season, seldom had any of their internodes more than half an inch long.

The different degrees of shortening of the internodes determine the height of the affected plants and other differences in appearance that result from having the foliage dense or open. The shortening and irregularity of the internodes are features of crazy-top that should be considered in relation to the brachytic or "cluster habit" of some varieties of Upland cotton, as already noted in the review of brachysm.

#### ABNORMAL LEAF-FORMS

Reduction and deformity of leaves are not prominent features of crazy-top in Pima cotton, though often very distinct in Upland cotton, especially under stress conditions. Some of the distortion that occurs with crazy-top may be ascribed to tomosis, which frequently accompanies crazy-top, especially in Upland cotton, under adverse conditions, but the leaf-forms may be very abnormal with no indication of tomosis. It is only in extreme cases that the crazy-top deformities in Upland cotton may be equal or even greater than the cyrtosis deformities in China, though still exceeded by the injuries of Upland cotton in Haiti, associated with the stenosis disorder.

The extreme of crazy-top injury is shown in Plates 10 and 11 from a photograph of Hartsville cotton secured by Robert D. Martin in September, 1923, from badly affected areas that had been located by C. J. King in the field of Hartsville cotton near Casa Grande, Ariz. (See also Pl. 1.) At that time some of the plants had the leaves of the upper portion of the stalk reduced to mere rudiments or quite abortive. In that condition there was a notable similarity to the smalling disease in Haiti, as shown in Plate 9. The resemblance was recognized at once by A. T. Valentine, who reached Arizona in August after seeing the Haitian disease in July. Thus the similarity of the symptoms of the two diseases in Upland cotton led to a more detailed comparison of other features of crazy-top with the disorders that had been studied previously in Haiti and in China.

Even when there is little or no distortion of the crazy-top leaves in Upland cotton, the size of the leaves may be greatly reduced, and the form of the leaves may be changed by broadening and shortening of the lobes, so that the outlines are much more rounded. (See Pl. 12 and 13.)

Some plants have much less reduction of leaves than others, though the leaves are of simplified shape, with the lobes only slightly developed. On the other hand, some plants have leaves very small, but without showing any tendency to reduce or suppress the lobes. Hence it may be inferred that the size and the shape of leaves are separate "characters." In some of the late season growth as shown in Plate 14 the leaves are aborted, so that the top of the plant shows only bracts, or bracts and small flowers, sometimes in a dense cluster or a spike, with a complete absence of leaves.

With Pima cotton there is a less obvious change in leaf-forms, but small simple leaves, as shown in Plate 3, are of more frequent occurrence on small crazy-top plants, at least where growth is restricted by less favorable cultural conditions. Thus the restriction of growth and the reduction of many of the leaves to simple form is in common with Upland cotton.

#### LEAVES NOT DISCOLORED

The lack of mottling or discoloration of the leaves is a peculiar feature of crazy-top, in view of the similarity in other respects to mosaic diseases. Even where the affected plants appear stunted and show many deformities of the leaves or other parts, the foliage is not discolored, and the crazy-top areas appear darker than the rest of the field at the end of the season, possibly because the plants are sterile and the foliage does not ripen as on the normal fruitful plants. However, it is known that other mosaic symptoms may occur without mottling of the leaves. In a mosaic disease of the potato, as reported recently by Mac-Millan (15), the mottling of the leaves does not appear in plants that are grown at high altitudes, 8,000 to 8,500 feet, in the Rocky Mountains. The more intense sunlight of the high altitudes was found to be responsible for the disappearance of mottling, as proved by an experiment with plants grown in partial shade, which showed the usual discoloration of the leaves.

A recent account of a growth disorder of strawberries describes the leaves and inflorescences as greatly reduced and distorted, with no indication of mosaic discoloration, although under some conditions a marked reddening of the surfaces may appear. This disorder is supposed to be due to nematodes that are found in the terminal buds of the abnormal strawberry plants (1).

Though the color is not altered, the texture of the leaves in crazy-top appears to be slightly firmer and more brittle. A difference of behavior was noted by Robert D. Martin in crazy-top material collected and photographed in September and placed in water to keep fresh. It was noticed that the crazy-top growth did not revive, but soon shriveled and dried, although the stems were standing in

water and the normal foliage below the crazy-top growth revived completely and remained fresh for several days.

If any difference in color is to be detected, the foliage of the crazy-top plants appears to be darker or to hold its green color late in the season. The darker foliage could be considered as a result of sterility, rather than as a character of the disease, but confirms the absence of any mosaic effect. If a color difference had been shown the disease undoubtedly would have been considered as a mosaic as soon as its existence was recognized.

#### SHEDDING OF FLORAL BUDS

Abortion of the floral buds or "squares" is a feature of crazy-top, as of cyrtosis and stenosis, with the extent of abortion and the stage at which it occurs depending very largely upon the external conditions and also showing a wide range of individual variation among neighboring plants when the critical conditions are approached. All stages of sterility may be shown, from plants that shed all of their floral buds at very early stages through those that retain their buds to larger sizes, with some that produce flowers but fail to set any bolls and others that set bolls, but still differ in numbers and degrees of development attained.

Even at the end of the season when most of the plants are showing more normal behavior some individuals may remain completely sterile and fail to develop any of their squares beyond the microscopic or scarcely visible sizes. (See Pl. 12.)

#### ABNORMAL INVOLUCRES

Some of the crazy-top plants have very small involucre bracts, even on the buds that are retained to the stages of flowering and fruiting (Pl. 14). The marginal teeth of the bracts may be very small, or very irregular in size, or deeply split into three lobes, as in the abnormal involucres that are of frequent occurrence in brachytic varieties, representing intermediate stages between bracts and leaves. Some of the reduced bracts of crazy-top plants have only three teeth, like the small involucres of *Thurberia thespesioides*, a plant related to cotton, growing wild in the mountains of Arizona.

Involucres formed of a single empty bract, resulting from abortion of flower-buds at very early stages, occur frequently on the crazy-top growth of Pima and Acala cotton. Similar sterile involucres were abundant in weevil-infested fields at San Antonio, Tex., in 1921 (?). The production of such involucres was considered as an abnormal condition that might be due to persistent pruning of the floral buds by boll weevils, though it now seems possible that a growth disorder might be involved, in addition to weevil pruning. Other indications of a growth disorder in south Texas are reported in a recent letter from B. V. Hasselfield.<sup>6</sup>

<sup>6</sup> An extensive shedding of small squares at the U. S. Experiment Farm, San Antonio, Tex., is also reported by Robert D. Martin under date of June 19, 1924, with specimens of an insect that has been identified by the Bureau of Entomology as *Psallus seriatus* Reuter, the so-called "cotton flea." Most of the squares were being shed at very early stages of development, between 1 and 5 mm. in diameter, so that relatively few buds reached the stage of being infested by the boll weevil.

The popular belief in the relation of the "flea" to the shedding of the squares has been questioned because the insects are found where the severe shedding does not occur, and because shedding has continued where the insects have been kept away from the plants by cages. But, with a growth disorder, shedding would continue without the insects, or the disease might be absent while the insects were present. A disease conveyed by the "fleas" or other insects is indicated by the continued shedding of the young buds and the formation of many ablastic involucres, as observed at San Antonio in 1921; but the striking deformations of the leaves and branches produced in Upland cotton by the crazy-top disorder in Arizona apparently do not occur in Texas. For a disorder manifested chiefly by the shedding of the squares, *ablasty*, or square-drop, would be an appropriate name. More frequent over-wintering of infected cotton plants or native malvaceous weeds might explain the restriction of the disease to southern Texas, although the insect is more widely distributed. Such a disease may be preventable if the source of infection can be determined.

## ABNORMALITIES OF THE FLOWERS

Most of the flowers that were produced on the late top growth of Hartsville cotton near Casa Grande were very small and the anthers did not open. Many of the anthers were shrunken, as though the pollen had not developed. A recent period of cold rainy weather might explain the failure of the anthers to open, but the failure of the pollen-grains to develop may be associated with crazy-top.

A case of complete sterility was observed in 1922 in the Coachella Valley, between Indio and Palm Springs, in a field of an okra-leaf strain of Acala cotton isolated by several miles from any other cotton, which may represent an analogous case of pollen-suppression by a growth disorder. A careful examination of many flowers in different parts of the field showed that no normal stamens were being developed, and that all of the anthers failed to open, although there were many flowers.

Earlier in the season a few bolls had been set on some of the plants, but widely scattered through the field. This might indicate that the disease began at the early flowering stage, or that some of the plants were not stricken as soon as others, as might be the case if a growth disorder were present. In view of the isolation of the field from other cotton any infectious disease must be supposed to have come from the native vegetation. A partial deficiency of pollen has been observed also in a planting of Acala cotton in another mountain valley near Palm Springs, confined to individual plants, which might result from a scattering infection with a growth disorder.

The reduction in the size of the flowers at Casa Grande was not regular, nor in definite proportion to other symptoms of the disease. In some cases rather large flowers were produced on badly distorted and reduced growth. Even on normal plants the size of the flowers may be somewhat reduced toward the end of the season.

## DWARFING OF BOLLS

Though plants that are badly affected with crazy-top became completely sterile, under the stress conditions of the summer months, there is a partial recovery or mitigation of the symptoms under more moderate late-season conditions. In the crazy-top areas of the Hartsville field at Casa Grande only a small proportion of the plants recovered to the extent of retaining and developing bolls, and most of the bolls in such cases were very small and misshapen (Pl. 15).

In Pima cotton the late season return to a fruiting condition is more general and the bolls are more normal in shape and size, though fewer seeds are developed than in the late-season bolls of adjacent plants that are not affected with crazy-top. Data recorded by Robert D. Martin on numbers of seeds and abortive ovules of bolls from 25 crazy-top plants and 25 normal plants of Pima cotton, grown at the Sacaton, Ariz., seed-farm in 1923 are given in Table I.

Smaller numbers of good seeds were found in the bolls of the late-season crazy-top growth than in the earlier or mid-season bolls of the same plants, and both classes of bolls of the crazy-top plants had notably fewer seeds than the corresponding classes of bolls from adjacent plants not affected by crazy-top. The reduced number of ovules in the crazy-top bolls may not be significant, except as an indication that the ovules aborted at an earlier stage so that the rudiments did not persist in visible form in the mature bolls. Some of the "middle crop" bolls, though borne on apparently normal fruiting branches below the crazy-top growth, probably were produced during the crazy-top period, and thus may have shared any adverse influence of the disease.

TABLE I.—*Comparison of seeds per boll from crazy-top and normal plants at the United States Field Station, Sacaton, Ariz., 1923*

Bolls from "crazy-top" Pima plants						Bolls from normal Pima plants							
Plant No.	Top crop bolls			Middle crop bolls			Plant No.	Top crop bolls			Middle crop bolls		
	Good seeds	Aborted seeds	Total ovules	Good seeds	Aborted seeds	Total ovules		Good seeds	Aborted seeds	Total ovules	Good seeds	Aborted seeds	Total ovules
1.....	18	0	18	18	1	19	1.....	8	10	18	19	2	21
2.....	12	5	17	16	5	21	2.....	19	2	21	21	0	21
3.....	9	11	20	14	5	19	3.....	20	1	21	19	2	21
4.....	9	10	19	14	7	21	4.....	17	1	18	20	0	20
5.....	16	2	18	18	3	21	5.....	19	2	21	18	3	21
6.....	10	6	16	15	6	21	6.....	15	3	18	19	2	21
7.....	10	9	19	17	3	20	7.....	19	2	21	17	3	20
8.....	9	8	17	13	7	20	8.....	15	1	16	20	1	21
9.....	9	11	20	17	3	20	9.....	19	0	19	20	0	20
10.....	9	6	15	12	11	23	10.....	19	2	21	17	2	19
11.....	15	3	18	23	0	23	11.....	9	10	19	18	2	20
12.....	7	11	18	13	4	17	12.....	14	2	16	19	2	21
13.....	11	4	15	17	2	19	13.....	17	4	21	16	4	20
14.....	11	6	17	10	10	20	14.....	18	4	22	18	3	21
15.....	11	8	19	11	8	19	15.....	16	2	18	16	4	20
16.....	14	3	17	15	4	19	16.....	19	2	21	21	1	22
17.....	14	4	18	17	4	21	17.....	11	6	17	19	2	21
18.....	13	7	20	19	3	22	18.....	15	6	21	19	1	20
19.....	15	3	18	15	3	18	19.....	21	0	21	14	7	21
20.....	14	4	18	15	5	20	20.....	19	2	21	18	4	22
21.....	6	10	16	20	4	24	21.....	7	12	19	19	2	21
22.....	11	7	18	18	5	23	22.....	15	6	21	17	4	21
23.....	13	3	16	19	0	19	23.....	19	2	21	19	2	21
24.....	11	5	16	15	6	21	24.....	20	0	20	19	2	21
25.....	8	10	18	15	6	21	25.....	19	2	21	17	2	19
Total...	285	156	441	396	115	511	Total...	409	84	493	459	58	517
Average.	11.4	6.24	17.64	15.84	4.6	20.44	Average.	16.36	3.36	19.72	18.36	2.32	20.68

## IRREGULAR OCCURRENCE OF CRAZY-TOP

The occurrence of crazy-top in the fields is as irregular as could well be imagined. Though a general tendency to appear in spots must be recognized, the margins of the spots are not definite, but show affected and normal individuals mixed indiscriminately in the rows. Isolated crazy-top plants may be widely scattered or in small open groups with normal individuals between. Striking contrasts appear between adjoining rows, when many plants in one row are affected with crazy-top and the next row is entirely free from the disorder.

If the soil or the cultural conditions were responsible for inducing the disease, more definite spots would be expected, with the marginal plants of the larger spots showing a series of gradations in the extent of reduction and deformity of the plants. Also some relation would be expected between the occurrence of the disease and the general growth conditions that determine the general size and vigor of the plants. But such relations are conspicuously absent. The occurrence of the disorder includes a very wide range of soil conditions, and plants of all sizes and degrees of vegetative vigor are affected.

The fact that badly affected individuals are so often scattered indiscriminately among plants that have continued the normal habits of growth through the season is also adverse to the idea of a resident cause of the injury located in the soil. It may be inferred from the failure to spread that the cause of the disorder, or the agency of distribution, operates in a very sporadic and temporary manner. Otherwise the infection would not remain scattered or sporadic but would be complete and continuous by the end of the season as was the case with

the club-leaf disorder in China, where all of the plants became affected in the latter part of the season, and all of the late-season growth was abnormal.

The irregular and interrupted distribution of crazy-top would seem to indicate that the disease is spread by insects or other agents that are not continuously present in the fields, or all of the plants would be affected, as in the cyrtosis disorder in China. The distributor may be active in the spring and fall but not in midsummer, as with the plant lice which cause the leaf-curl disorder.

In the Hartsville cotton field at Casa Grande some of the plants had been dwarfed at very early stages and were only 6 or 8 inches high at the end of the season. Around these were other badly crippled plants, but of larger size, 15 to 18 inches high. Finally, there were many plants that had grown to full size, with a height of 3 to 4 feet, and produced normal bolls, although the late-season top growth was definitely abnormal. Thus it seemed that three stages or periods of infestation might be indicated in this field.

The irregular mode of occurrence of crazy-top also appears inconsistent with the idea of genetic or physiological causes. A genetic defect would be scattered generally through the fields in the same way as other off-type individuals, or "rogues," while physiological effects would be expected to show more definite grouping and more continuous gradations, but the facts do not accord with either of these assumptions. While crazy-top appears under a wide range of conditions, there are many fields that show no crazy-top.

In order to test heritability of the disorder, seed was saved in 1923 from individuals having pronounced symptoms of crazy-top, and progenies are being grown in 1924. Also seed was saved from scattered rogues or off-type plants of the Pima, Acala, and Hartsville varieties, and the progenies are to be studied with a view to the possible relations with crazy-top.<sup>7</sup>

Of course it is not possible to have a clear understanding of the distribution and occurrence of such a disease until the causes are discovered, including the sources of infection and the insects or other agents for conveying the virus from one plant to another. The usual plant lice of cotton may be the conveyors of crazy-top, or leaf-hoppers, thrips, mites, or nematodes may be implicated.

### CRAZY-TOP UNDER DIFFERENT CONDITIONS

The disorder is not restricted to a particular type of soil or to other special conditions, though the severity of the symptoms and the extent of injury depend largely upon the conditions. The occurrence of the disorder must not be confused with the extent of injury. If only the extreme cases were noticed one might suppose that the disease appeared only with bad conditions, but crazy-top plants are also found under the most favorable conditions, where the plants that are not affected show the most normal development and produce the largest crops. It should be remembered that shedding, sterility, and abnormalities of branching also occur in plants that are over-luxuriant; and that such symptoms may be induced by alternate checking and forcing of growth, as may occur in rich soils which are too heavy to take water readily or have too light a subsoil to hold water from one irrigation to another. Under such conditions the stalks may continue to grow though most of the fruiting branches are aborted.

If no extreme conditions were encountered, the recognition of such a disease would be the more difficult, since the effects might be limited to a slight depression or subnormal status of the affected plants, scarcely to be distinguished from

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<sup>7</sup> A report from C. J. King, under date of June 12, 1924, shows that 132 hills were planted at Sacaton with seed from 14 crazy-top plants of Hartsville cotton, but only 3 hills germinated, all from the seed of one plant. Of Pima cotton, 140 hills were planted with seed from 13 abnormal individuals, and germinations were secured in 86 hills. Only 4 lots of the Pima seed showed no germinations.



the slight constitutional weaknesses, like those that may be detected when progenies are compared in plant-breeding work. With the existence of such disorders recognized, their relation to the problems of adaptation must be considered.

Though the occurrence of crazy-top may have no relation to the conditions that determine the growth and productiveness of the normal plants, it may be a serious factor in determining the values of varieties for purposes of production. The presence of a slight disorder, as of crazy-top in Pima cotton, under moderate conditions, might remain unrecognized, although rendering the crop much more sensitive to unfavorable conditions. The effects of the disorder would be considered as a character of the variety, which would appear less suited to cultivation, as when crazy-top was looked upon as a special weakness of Pima cotton. In the same way, it might be supposed that a field, or a farm, or a section of country was not well suited to cotton, while the crop in reality might be suffering from a growth disorder rather than from any unfavorable soil conditions.

If such a disease were carried over from year to year by survival of infested plants through the winter, the yields would be reduced and the necessity of a more frequent rotation with other crops would be inferred. The rotation might be desirable for other reasons, and less crazy-top might be encountered in a different field. It is reported that some of the worst cases of crazy-top have occurred on new land, but of course this does not prove that affected areas do not increase where cotton is grown continuously.

#### CRAZY-TOP IN DIFFERENT VARIETIES OF COTTON

The differences in crazy-top symptoms between the Pima and the Upland varieties are in line with what is known regarding the varied degrees of susceptibility of different types of cotton to other growth disorders. The theory that crazy-top is due to a special weakness or tendency to degeneration in Pima cotton must be discarded, in view of the fact that the Upland varieties are more seriously affected than the Pima, especially when grown under unfavorable conditions. The more extreme symptoms that appeared in the field of Hartsville cotton at Casa Grande have not been observed in Pima.

Near Phoenix, Ariz., the symptoms of crazy-top were observed in adjacent plantings of Pima and Acala, under conditions of luxuriant growth, and there the disease reactions were more similar than would be inferred from the contrasts that had been shown between Pima at Sacaton and Hartsville at Casa Grande. The characteristic crazy-top symptom in Pima, the strong upright branches at the top of the plant, had appeared to be lacking in the Hartsville variety at Casa Grande, but was shown quite distinctly in the Acala at Phoenix, though somewhat less pronounced than in the adjacent Pima rows. In this planting of Acala the growth of the main stalk was but little restricted, and the tendency to reduction and distortion of the leaves was much less apparent than in the Hartsville planting at Casa Grande, though in some plants it was definitely shown. Also many of the Acala plants had a tendency to abort the leaves near the top of the main stalk, thus forming tufts or spikes of very short branches bearing small squares, as shown in Plate 14.

Also there was noted in Acala the same tendencies as in Pima to transform fruiting branches into vegetative branches, or to develop vegetative shoots from axillary buds of fruiting branches, with or without suppression of the terminal buds.

In China the Upland cottons were more affected by the cyrtosis disorder than were the Egyptian and Sea Island cottons, but the native Chinese cottons, representing the Asiatic type, were more strikingly affected than the Upland



varieties. Yet one variety of Asiatic cotton, from India, growing at an experiment station near Wuchang, showed none of the club-leaf distortion, although the adjoining rows of native Chinese cotton were badly affected. This indication of the existence of an immune variety of Asiatic cotton is confirmed by information received recently from China by J. B. Griffing of Nanking University, with the photograph shown in Plate 8.

In Haiti the Upland cotton was more deformed by the stenosis disorder than Sea Island cotton. The so-called "Native Haitian" or Bourbon cotton showed no injury from stenosis, while the Upland cotton showed more serious injuries than from cyrtosis in China.

### INDIVIDUAL DIVERSITY OF CRAZY-TOP PLANTS

A general impression of uniformity may be given by the reduced growth of the plants in a crazy-top spot, like that shown in Plate 1, but closer inspection shows many differences among the adjacent individuals, in the size, shape, and distortion of the leaves, in the occurrence of tomosis, in the shedding or retention of the squares, in the forms and positions of the involucre bracts, in the attainment of the flowering stage, and in the development of bolls. (See Pl. 2, 10, 11, 12, 13, 14, and 15.) The diversity may be comparable to that of a hybrid stock, or to the wide range of differences that sometimes appears in a stock that is being grown under new or unwonted conditions.

Many stages and degrees of abnormality had been observed in the Chinese mosaic disorder, and a still wider range of diversity among the affected plants was a feature of the stenosis disorder in Haiti, and a similar diversity appears in crazy-top. Though the individual differences are less striking in Arizona than in Haiti, they are obvious enough, especially in the Upland varieties, as will be seen from the photographs of a few examples, in Plates 10 to 15.

Such differences might be ascribed to irregularity of cultural conditions, but this view is not likely to be held when the differences that appear in adjacent plants in a crazy-top area are contrasted with the greater uniformity of the normal plants nearby. Little doubt will remain that the diversity of crazy-top plants is abnormal, as well as the particular changes of characters.

### RELATION OF GROWTH DISORDERS TO HEREDITY

The individual diversities of plants affected with growth disorders show that many deviations from the normal hereditary course of development are induced. The normal characters are no longer brought into expression, but are replaced by aberrations or abnormalities of many kinds, differing among the types and varieties of cotton as well as among the individuals of the same stock. Different characters are shown, not because the plants have different conditions, but because development is disturbed by loss of the normal adjustments or expression relations that determine the characters or course of development of the individual. The changes of expression are somewhat definite, since otherwise the same aberration would not be repeated so consistently in the structural units of the individual plant, while neighboring individuals of the same stock show equally consistent expressions of other abnormal features.

Thus the growth disorders may be of special interest to students and investigators of heredity, as showing that the expression of the characters of plants may be changed by chemical compounds in a manner that may be analogous to the recently discovered control of development in animals by secretions of the ductless glands. Abnormalities that otherwise would be considered as aberrations of heredity become explainable as effects of poisons or of disturbances of the normal sequence in the chemical reactions that attend the process of development.

Even if organisms were found to be responsible for the growth disorders, the abnormalities would still be ascribed to the secretions or waste products of such organisms. Of course, it has long been known that insects could produce galls and other localized malformations, but in the growth disorders the whole subsequent development of the plant may become abnormal. Instead of a merely local irritation, as in a gall or a hexenbesen, the normal course of development may be lost suddenly, at any stage in the growth of the plant. (See Pl. 8, 9, 10, and 11.)

The growth disorders afford additional illustrations of the fact that two distinct processes are involved in heredity. The expression or development of the characters in the individual is distinct from the transmission of the characters. How the changes of expression are brought about is still unknown, but it is plain that expression can be modified, and in many different ways, by the growth disorders, and that the changes may be permanent during the life of the individual organism.

It has long been recognized that mutilations or defects induced by diseases are not inherited, but such accidental injuries are not the same as the changing of the course of development by the growth disorders, which continues through the life of the plant individual. Except that all of the later growth is abnormal, the changes of characters in crazy-top are similar to those that occur in "bud mutations," where single branches show changes of characters that are definite and can be propagated as a distinct variety. Also, in such cases the original characters are not lost, since reversions to the form of the original variety may occur in later bud mutations.

It has been customary to assume the existence of a "mechanism of heredity" to account for the very definite transmission of characters, but less consideration has been given to the idea of a mechanism of expression of the characters, as distinct from the mechanism of transmission. Definite ideas of such "mechanisms" are still lacking, but it is plain from the growth disorders that the control of development may be changed profoundly and in ways that may interfere quite definitely with the development of the individual, without corresponding changes of the transmitted characters. Though the causes are still unknown, the growth disorders undoubtedly have this remarkable property of interfering and altering the adjustment of the mechanism of expression.

Although the normal hereditary characters are thrown out of expression in the affected plants, they reappear in the next generation. The disturbing cause, that in some way prevents or masks the expression of the characters, apparently is not carried in the seed in most of the growth disorders, though some are difficult to distinguish from genetic defects. Yet the abnormal expression of the characters, as induced by the growth disorders, may go on indefinitely in the vegetative or somatic tissues of the plant, in all of the new growth that is formed. Some of the mosaic diseases are communicated readily by grafting or by artificial infection, as well as by insects or other natural carriers.

As yet there is nothing to show how the expression of the characters is deranged. Even the normal processes of growth are little understood, and we have no conception of what it is that controls the development of the plant and brings the normal inherited characters into expression. Though each of the growth disorders shows a general trend of abnormality in one direction or another, the greater individual diversity of the abnormal plants would seem to indicate a negative effect or loss of the normal adjustment of the expression relations of the characters, rather than a positive effect of establishing a new determination of the characters, although a certain stability is shown in the expression of the abnormal characters.

## RELATION OF GROWTH DISORDERS TO COTTON BREEDING

The reactions of varieties to the growth disorders undoubtedly must be considered as factors of adaptation. Susceptibility or immunity to a disorder might easily determine the success or failure of a variety in any district or region of production. Some of the types apparently are completely immune to disorders that in other types cause extensive and destructive malformations.

The changes that may be caused in the characters and appearance of the plants by even the less serious disorders will need to be understood by those who take part in the breeding of varieties and the roguing of seed-stocks. The sporadic occurrence of such a disorder as crazy-top in scattered individual plants would give much the same impression as a mixture of varieties or a frequency of rogues or off-type plants, and might lead to the rejection of a superior seed-stock in the belief that it had "run out" or had been allowed to cross with other varieties. The growth disorders may explain why fields of cotton raised from the same stock of seed may sometimes show widely different degrees of diversity.

Other disorders may exist, less frequent or less definite than crazy-top, and bring serious complications into the work of the breeder. The occurrence of such disorders might be very irregular, not only sporadic as to place, but also intermittent in time or seasons. Conditions that had no relation to the growth of cotton might determine the existence of such diseases in malvaceous weeds or in other wild plants of adjacent lands, while other conditions would determine the presence or absence of insects to carry the disease from the native plants to the cotton.

In bulk plantings an intermittent growth disorder might be confused with a Mendelian recessive character, which would not appear in the first generation of a cross, but would recur in the second and later generations. A disorder that did not reappear in the second generation might be considered a mass variation. Such outbreaks of brachysm, fasciation, or leaf-deformities are sometimes reported, but generally are disregarded by investigators, for lack of any rational explanation. In selection and roguing work with cotton it has been observed that two or more of the off-type plants are likely to be found together or close at hand, instead of being scattered indiscriminately through the fields. While seeds of the same boll might remain together, such tendencies toward grouping of the abnormal plants should be considered as indications that growth disorders may be involved.

Study of aberrant plants that occurred in spots might lead to a recognition of other scattered plants as representing the same type of abnormality, allowance being made for the diversity that often exists among the individuals affected by a growth disorder. Also it is possible that growth disorders may have very mild or scarcely appreciable symptoms under moderate weather conditions early in the season, and that the serious distortion symptoms may appear abruptly at a later stage, when stress conditions occur.

## SUGGESTIONS FOR FURTHER STUDY

With the recognition of crazy-top as a growth disorder, several new lines of investigation are suggested. The increasing substitution of Upland cotton for Pima in the Salt River Valley may facilitate the investigation of crazy-top, as well as increasing the importance of the disease, because the injuries are more severe in Upland cotton. Careful comparisons should be made where Pima and Upland cotton are affected by crazy-top under the same conditions. In view of the indications of more serious injury to the Upland plants, it is important to

determine whether the yields are reduced in Upland cotton to a greater extent than in Pima.

Locating and mapping of crazy-top areas would be desirable, to determine whether the disease recurs regularly in the same places. Also such localizing of the disease might lead to the discovery of native plants or weeds that carry the disease through the winter and serve as reservoirs of infection. There are many wild Malvaceae in Arizona, and crazy-top might not be confined to a single species. A wider range of host-plants is indicated for mosaic disorders than for fungous or bacterial diseases. The sugar cane mosaic is communicable to grasses of other genera, and an inoculation of *Nicandra physaloides* with the potato mosaic has been reported recently (9).

Insect carriers of the crazy-top infection might also be identified, if the disease were traced to wild plants, and such knowledge might make it possible to control the disease by removing the source or preventing infestation. On the other hand, if the sources and agents of infection are neglected, the disease may increase through the establishment of more centers of infection among the wild plants.

Over-wintering of crazy-top plants should be studied, to determine whether such plants survive and carry over the disease, whether the symptoms can be detected early in the season, and whether such plants are a factor in extending the disease.<sup>8</sup>

Fields that have had crazy-top injuries in previous years should be watched carefully to see if the beginning of the injury can be detected. It is believed by some observers that the onset of the disease is definitely indicated by the shedding of all of the squares and young bolls. Where abnormal shedding occurs with no apparent cause, the development of other crazy-top symptoms should be looked for, although it is possible that infestation may occur at earlier stages, and that the more striking symptoms are developed when stress conditions are encountered.

Early infestation may be indicated by different habits of growth of the plants or more gradual development of the symptoms, without the very abrupt change that apparently marks the onset of the disease in previously normal individuals. The occurrence of large numbers of such plants, though without the usual crazy-top appearance, should be studied with reference to early-season infestation of crazy-top, which might render the growth more consistent, without the abrupt changes that result from infection at later stages of development.

Cases of delayed recovery from the hybosis disorder have been noticed as occurring in groups or areas like crazy-top and possibly may be connected with early transfers of crazy-top infection from native vegetation by the plant lice. Also early infestation in Pima cotton may be indicated by plants of slender upright habit that often are completely sterile, although a few bolls may set late in the season. If early infections of crazy-top could be recognized and destroyed, protection might be secured against later spreading of the disease. Or if large areas are found to be affected, the cotton could be plowed up and the land used for other purposes.

<sup>8</sup> Persistence of the disease by over-wintering of affected plants was definitely shown in the Hartsville field at Casa Grande, visited May 27, 1924. The abnormalities of the new growth were as striking as those of the previous season, and appeared on many plants which had not shown the disease in the fall, along the margins of the affected area. In a field of Pima cotton near Scottsdale, visited May 29, the shoots of many plants also showed a definite reduction and cupping of the leaves, although the symptoms generally were much less pronounced than in the Hartsville field, and many of the Pima plants appeared normal. Crazy-top symptoms on seedling plants were first noted by C. J. King on June 11, 1924, in the Hartsville field adjacent to the diseased plants that had survived the winter. Since the field was being abandoned as unproductive, and was in a district well isolated from other cotton, Mr. King had arranged with the owner to try the experiment of over-wintering the diseased plants and of planting fresh seed in a part of the affected area.

\* Protection and shading of some of the affected plants would determine the extent to which the crazy-top symptoms are modified by the condition of growth, and whether mosaic discolorations can be induced by shading, as in the case of the potato mosaic under high-altitude conditions. By means of insect-proof cages, the relation of the disease to insect carriers may be demonstrated, or plants inoculated artificially to study the conditions of infection.

### CONCLUSIONS

Abnormal behavior of cotton plants, resulting in partial or complete sterility of the affected individuals, is of frequent occurrence in Arizona, and is known among the farmers as "crazy-top." The name *acromania* is suggested as a technical designation for the crazy-top disorder, both names alluding to the abnormal branching in the upper portion of the plant, which is a striking feature of this disorder. The recognition of *acromania* as a distinct disorder has resulted from previous study of other disorders which are to be compared and distinguished from *acromania*.

Crazy-top has been ascribed to exhaustion of the soil and to a supposed "running-out" of the Pima variety, but these theories are plainly inadequate. Although the crazy-top symptoms are more striking and the injuries are greater where the cultural conditions are less favorable for the plants, the disorder is not restricted to Pima cotton or to particular types of soil, or to lands that have grown cotton in previous years.

The crazy-top injuries are more severe in Upland cotton and the symptoms more numerous. In addition to the abnormal branching and sterility, as in Pima cotton, a wide range of diversity is shown in crazy-top plants of Upland cotton, with many forms and degrees of reduction and distortion of the leaves, involucres and floral organs. Also, the Upland cotton apparently does not recover or return to more normal behavior late in the season to the same extent as Pima.

The reduction and distortion of the leaves and complete sterility of the top growth in Upland cotton are similar to the cyrtosis and stenosis disorders in China and Haiti. Hence a comparison of crazy-top symptoms with those of other growth disorders, including the mosaic diseases, seems justified, although the characteristic feature of mosaic diseases, the mottled discoloration of the leaves, is not present in crazy-top. The less striking effects of crazy-top in Pima cotton, as well as the variation of symptoms and injuries under different conditions, also are paralleled in cyrtosis and stenosis.

Although the late-season growth of affected plants is less abnormal than the growth at mid-season, crazy-top apparently is a disorder resulting in permanent injury like cyrtosis and stenosis, instead of a disorder causing temporary injury like tomosis and hybosis.

The change in the branching habit results from the fact that the fruiting branches of the upper part of the plants are partially or completely transformed or replaced by vegetative branches. Partial transformation is the more frequent condition and is shown by the abortion of the floral buds at very early stages, leaving a minute bud scar on each internode of the branch.

Association of the crazy-top disorder with the mosaic diseases is suggested by the similarity of symptoms and modes of occurrence, and further knowledge may lead to the discovery of measures of restricting the injuries. The variable and sporadic occurrence of the disease may be explained if it is found that the infection is carried by insects from some of the native wild plants or weeds that are related to cotton. By determining the source of infection it may be possible to protect the cotton fields against the crazy-top disorder, or to avoid the injurious effects in other ways.

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# PLATE 1

An area affected with crazy-top in a field of Hartsville cotton near Casa Grande, Ariz., at the end of the season. The sterility of the crazy-top plants is indicated by the lack of open bolls in the affected areas. Also sterile crazy-top plants are shown at the right of the foreground.

(828)







## PLATE 2

A. Crazy-top in Pima cotton, showing the characteristic appearance at the end of the season, the plants sterile below but with an excessive top-growth of stiff erect branches, with numerous buds and small bolls. at the end of the season. To compare with Pl. 2 B.

B. The same plants as in Pl. 2 A, with the leaves removed, showing more clearly the characteristic ascending branches of the upper part of the plant, and the nearly complete absence of mature bolls on the lower fruiting branches. Photographed by Robert D. Martin, Sacaton, Ariz., September, 1923.

### **PLATE 3**

**Late-season fruiting branch showing irregular shortened joints, reduced simple leaves and small bolls, caused by crazy-top in Pima cotton.**





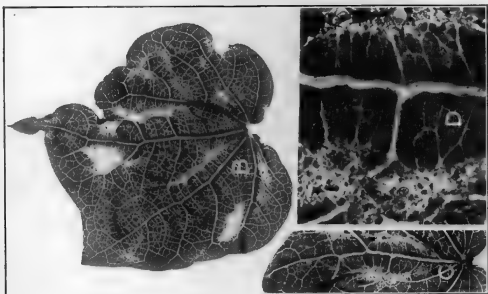
#### PLATE 4

A fastigate variety of Egyptian cotton called Dale, with suppression of fruiting branches comparable to brachysm in Upland cotton and to crazy-top in the Pima variety. Figure A, top of a Dale plant showing stiff ascending branches, similar to crazy-top in Pima cotton. Figure B, top of a Dale plant with complete suppression of branches. Figure C, tops of two Dale plants, with two short branches at most of the nodes.

## PLATE 5

A. Recovery from tomosis disorder. Seedling of Egyptian cotton at Bard, Calif., 1911, showing abrupt recovery of the normal leaf-form after severe tomosis injuries to several of the lower leaves. (One-half natural size.)

B, C, and D. Relation of oil glands to tomosis. Leaves of seedlings of Pima cotton showing tomosis in several stages, beginning with translucent spots around dead oil-glands. Figures B and C about natural size. Figure D a section from near the middle of figure C, magnified about 4 diameters.







## PLATE 6

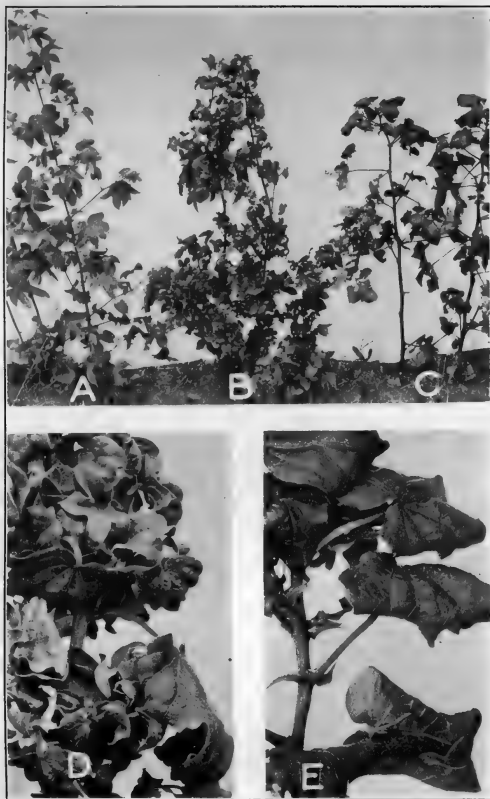
Hybosis in Pima cotton. Seedlings of Pima cotton with the first leaves above the cotyledons badly crumpled and deformed, but not perforated or mutilated as in tomosis.

## PLATE 7

Late-season leaf-curl (hybosis) of Upland cotton at Sacaton, Ariz., with shortening of internodes and crumpling of leaves, to be distinguished from crazy-top. (Natural size.)

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## PLATE 8

Cyrtosis disorder in China. A, Asiatic cotton from India, resistant to cyrtosis. B, native Chinese cotton affected with cyrtosis. C, Durango cotton affected with cyrtosis. D, Chinese cotton affected with cyrtosis, natural size. E, Durango cotton in China, affected with cyrtosis, natural size. Figures A, B, and C, from photographs by J. B. Griffing, Nanking University, Nanking, China, October, 1923.

## PLATE 9

Stenosis disorder in Upland cotton. Top-growth of two plants showing extreme forms of stenosis injury in Haiti, with abnormal branching, reduction, and distortion of leaves, and great diversity of affected plants, often with complete sterility in Upland cotton. To compare with extremes of crazy-top injury shown in Plates 10 and 11.







## PLATE 10

Crazy-top in Upland cotton. Abnormal branches of badly affected plants showing extreme reductions and distortions of leaves of Hartsville variety at Casa Grande, Ariz., September, 1923. Photographed by Robert D. Martin. (Natural size.)

## PLATE 11

Crazy-top in Upland cotton. Abnormal growth of badly affected plant showing suppression of fruiting branches and many stages and forms of reduction and distortion of leaves of Hartsville cotton at Casa Grande, Ariz., in September, 1923. Photographed by Robert D. Martin. (Natural size.)





## PLATE 12

Diversity in crazy-top plants. Top of a plant that was completely sterile, **all** of the flower buds being suppressed at very early stages, the leaves **greatly** reduced in size and with short broadly rounded lobes in comparison with **Plate 13**. (Natural size.)

PLATE 13

**Diversity in crazy-top plants.** Reduction of the size of leaves in Hartsville cotton without shortening of lobes, to compare with Plates 11 and 12 where the lobes are much broader and shorter.







#### PLATE 14

Diversity in crazy-top plants. Upper portions of two affected plants, showing differences in the production of leaves, in the retention of floral buds, in the shortening of the joints of the fruiting branches, and in the shape and size of the involucral bracts. In some cases the flower buds are retained and the leaves suppressed, as in the left-hand plant, or the growth may be more compact, forming a rather close spike of floral buds with no leaves. (Natural size.)

## PLATE 15

Bolls from crazy-top growth of Hartsville cotton, with normal late-season bolls of unaffected plants in the same field near Casa Grande, Ariz. (Natural size.) Most of the crazy-top plants shed all their flower buds or young bolls, but some of the affected growth produces small bolls at the end of the season.



# THE POLYEMBRYONIC DEVELOPMENT OF *PLATYGASTER VERNALIS*<sup>1</sup>.

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## INTRODUCTION

In a previous paper the writers (4)<sup>3</sup> referred to the desirability of demonstrating insect polyembryony in a species in which but a few individuals are developed from a single egg. The development of such a species was in part described for *Platygaster hiemalis*, a parasite of the Hessian fly.<sup>4</sup> It was found, however, that polyembryony was carried only to the point where twin parasites were produced from a single egg, thus demonstrating the simplest type of polyembryony possible.

A slightly more complex form of polyembryony will be described here for *Platygaster vernalis* (Myers), another parasite of the Hessian fly, in which an average of about eight individuals are developed from a single egg of the parasite. A knowledge of the development of this species will furnish a further clue to the more highly specialized forms of polyembryony in which as many as 150 to 2,000 individuals are produced from a single egg.

*Platygaster vernalis* develops only in the mid-intestine of the host larva. The development of a closely related species, *Polygnotus minutus* Lindemann, which is also confined to the mid-intestine of the Hessian fly larva in France, has been described previously by Marchal (5). Marchal's paper upon this insect is now difficult to obtain. However, his paper does not treat of the precleavage or cleavage stages of development in a sufficiently detailed manner to demonstrate polyembryony, but it does describe quite fully the organogeny of the embryos. There are also some indications that *P. minutus* and *P. vernalis* differ slightly in the details of their development, although it is difficult to determine this definitely, because the development of Marchal's species is not illustrated with sufficient histological preparations. In the present paper, therefore, emphasis has been placed upon a study of microtomic sections, and the paper has been illustrated<sup>5</sup> from this viewpoint rather than upon gross examinations.

## RELATION OF PARASITE TO HOST

The biology of *P. vernalis* with particular reference to its economic importance as a parasite of the Hessian fly, which is a serious pest of wheat, has been previously dealt with by the junior writer (2). The adults emerge from their

<sup>1</sup> Received for publication April 22, 1924.

<sup>2</sup> The writers gladly acknowledge the continued interest shown by Dr. L. O. Howard and W. R. Walton in the studies reported in this paper. To Doctor Howard is due the chief credit for calling the attention of American investigators to insect polyembryony. Our studies on the development of the *Platygaster* parasites of the Hessian fly were initiated at the direction of Mr. Walton.

<sup>3</sup> Reference is made by number (italic) to "Literature cited," p. 839.

<sup>4</sup> *Phytophaga destructor* Say.

<sup>5</sup> All of the illustrations of microtomic sections except figures C and D on Plate 1 were drawn by the senior writer. The remainder of the drawings were prepared by the junior writer. The photomicrographs were made by the photographer of the Bureau of Entomology.

cocoons (Pl. 1, E) during April and the early part of May. A group of approximately eight individuals emerge from each host puparium of the Hessian fly when parasitized by this insect. The female parasites, whether fertilized or unfertilized, deposit their eggs in the eggs of the host (Pl. 1, A, B) which are deposited on the wheat plants by the spring generation of flies. The parasite eggs begin development immediately (Pl. 1, C), the embryos being fully formed in the nearly mature host larvæ (Pl. 1, D) by the first of June. During June and July the parasite larvæ feed upon the contents of the host larvæ, each group of parasites devouring all of a host larva (Pl. 8, C) with the exception of its integument. After the parasite larvæ are fully grown (Pl. 8, B) they remain for some time in their cocoons which they have prepared inside the host larval integument. About the latter part of July the larvæ transform to pupæ (Pl. 8, D), these in turn transforming to adult parasites some time during August.

The adult parasites remain in their cocoons (Pl. 1, E) during the winter, being protected by the puparium or toughened integument of the host; and emerge from their cocoons and the host puparia in spring, by gnawing one or more holes through the cuticula and puparium of the host (Pl. 1, F). These adult parasites then search for eggs of the spring generation of the Hessian fly in which to oviposit.

#### PRECLEAVAGE DEVELOPMENT OF THE EGG

The eggs of *Platygaster vernalis* are always deposited singly, but occasionally a second or third egg may be placed in the same host egg by other females. However, the same female parasite usually avoids ovipositing in any host egg more than once. This parasite differs from *Platygaster hiemalis*, another parasite of the Hessian fly, therefore, in the number of eggs deposited at one time; *P. hiemalis*, as has been shown by the writers (4), placing a cluster of four to eight eggs in the host egg at each oviposition.

The egg of *Platygaster vernalis* is always so placed in the host egg that it is eventually found in the mid-intestine of the host embryo or young larva with unfailing regularity (Pl. 1, C). The egg does not and apparently can not undergo development in any other part of the host. The proper placing of the egg is doubtless accomplished by a complete coordination of certain factors, among which are the orientation of the host egg, the manner of striding the egg by the parasite, and the length of the parasite's ovipositor. It is of interest to note, on the contrary, that *P. hiemalis* always deposits its eggs in the host egg, so that they are never lodged in the mid-intestine, where apparently they would fail to develop.

#### THE NEWLY DEPOSITED EGG

The newly deposited egg is somewhat elongate, but it soon becomes more compact, cylindrical in shape, and rounded at both ends (Pl. 2, A), measuring, according to fixed and sectioned material, approximately 21  $\mu$  in length and 8  $\mu$  in width. The protoplasm of the egg appears very finely granular and uniform, and contains a spherical and darkly staining concentrated nucleus which measures 3  $\mu$  in diameter. Immediately after oviposition the nucleus is found in or near the center of the egg. The long and thread-like sperm (Pl. 2, B) is somewhat difficult to demonstrate in all eggs deposited by fertilized females, probably because of its wavy and spiral position in the protoplasm of the egg. There is also some evidence that not all eggs deposited by impregnated females are inseminated. The nucleolus or germ-cell determinant is wanting, just as it is in *Platygaster hiemalis*, and in *P. dryomyiae* as shown by Silvestri (8).

## MATURATION AND THE ORIGIN OF THE PARANUCLEAR MASSES

In a general way the maturation of the egg of *Platygaster vernalis* is similar to that described for other species of *Platygaster*. As far as can be ascertained, it is identical in fertilized and unfertilized eggs. As in previously described polyembryonic and some monembryonic Hymenoptera, the polar bodies are retained in the egg and eventually give rise to the paranuclear masses which have a nutritive function in the course of development of the embryos; while the oöcyte nucleus, whether fertilized or not, gives rise to the embryos.

The first maturation of the oöcyte nucleus is completed during the first eight hours after oviposition. Within the first 30 minutes the nucleus begins to expand, and by the fifth hour it is in the prophase stage of mitosis (Pl. 2, B, C). Thereafter maturation is completed quickly; the chromosomes being found first, grouped together at each end of the spindle but distinct from each other (Pl. 2, D), and then, later, somewhat concentrated into two separate nuclei (Pl. 2, E). The chromosomes of each of the two nuclei then condense further and form irregularly shaped, homogeneous, dark staining nuclei (Pl. 2, F, G), which are always quite conspicuous in the egg in spite of their small size. The first maturation takes place longitudinally in the anterior half of the egg, and results in the production of the first polar body, which passes to the anterior edge of the egg, and the oöcyte nucleus of the second order, which remains near the center of the egg.

Between the eighth and the twelfth hours after oviposition the first polar body and the oöcyte nucleus of the second order remain quiescent, but about the twelfth hour a second maturation of the oöcyte nucleus commences, and the mitotic division results in the production of two distinct groups of chromatin material in the central region of the egg (Pl. 2, H-J). The second maturation spindle is always considerably shorter than the first. Various stages of the division can be observed in eggs that are 12 hours old, indicating that second maturation is completed quickly. The anterior of the two centrally-disposed nuclei is the second polar body, which later migrates toward the polar or anterior region of the egg by the side of the first polar body. The posterior nucleus of the second maturation spindle becomes the female pronucleus of the fertilized egg or the cleavage nucleus of the unfertilized egg. It remains in the posterior half of the egg from the twelfth to the twenty-fourth hour, increasing in size during this interval from a diameter of  $2.2\ \mu$  just after maturation is completed to  $5.4\ \mu$  at the time of fusion with the male pronucleus.

About 24 hours after oviposition the two polar bodies begin to expand, becoming at first spherical and then oval (Pl. 2, K, L; Pl. 3, A, B), thus assuming the shape and appearance of typical paranuclear masses, as described for other polyembryonic Hymenoptera. As the polar bodies expand the chromatin breaks up into numerous small granules which are scattered throughout the plasma of each nucleus. As will be shown later, the polar bodies (henceforth known as paranuclear masses) migrate about in the egg and divide amitotically into similar secondary masses. It should be noted that simultaneously with the expansion of the polar bodies the egg increases in size, measuring in its greatest diameter when 1 day old approximately  $28\ \mu$  in length and  $9\ \mu$  in width.

## FERTILIZATION

During the first six hours after oviposition the sperm transforms into an oval nucleus (Pl. 2, D, E), which is always located in the posterior region of the egg. A stage of the male nucleus is then evident, when the chromatin resembles a tightly coiled thread (Pl. 2, F). The threadlike chromatin next breaks up into

numerous pieces (Pl. 2, I), which are then distributed over a matrix (Pl. 2, G, H). Meanwhile, the nucleus has expanded until it measures approximately  $5\ \mu$  in diameter. These changes undergone by the male nucleus are not always synchronous with the various stages of maturation but they take place between the tenth and twelfth hours after oviposition. From the twelfth to the twenty-fourth hours the male nucleus remains in a quiescent condition.

Fusion of the male and female pronuclei is effected about 24 hours after oviposition, in the posterior half of the egg (Pl. 2, K, L; Pl. 3, A, B). The egg now contains two paranuclear masses in its anterior region, and a cleavage or embryonic nucleus in its posterior region.

#### DIFFERENTIATION OF TROPHAMNION AND EMBRYONIC REGION

Shortly after the cleavage nucleus is formed, an area is seen to encompass it which is somewhat less dense than the remainder of the egg. This more lightly stained area is the embryonic region (Pl. 3, B), which together with the cleavage nucleus gives rise to the embryos. The remainder of the egg containing the two paranuclear masses constitutes the trophamnion.

The egg is henceforth properly known as the parasite body, for it now begins to increase in size. The active feeding of the host, which is soon to commence, permits the trophamnion to absorb the elements from the chyle in the host which are necessary for further growth. Similarly, the elaboration of the trophamnion and the distribution of the paranuclear masses within it are conducive to cleavage and the development of the embryos.

#### SUMMARY AND DISCUSSION OF PRECLEAVAGE DEVELOPMENT

In most respects the precleavage development of *Platygaster vernalis* is not unlike that previously described by the writers (4) for *P. hiemalis*, nor even greatly different from that described by Silvestri (8) for *P. dryomyiae*, which develops monembryonically. In *P. dryomyiae* the first polar body divides during the second maturation of the oöcyte nucleus, and the second polar body, produced at second maturation, unites with the posterior half of the divided first polar body to form one paranuclear mass; while a second paranuclear mass is developed from the anterior half of the divided first polar body. In *P. hiemalis* the writers have shown that the two polar bodies, resulting from the two maturations of the oöcyte nucleus, unite to form one polar nucleus, which later divides to form two subequal paranuclear masses. It has been shown above that in *P. vernalis* the two polar bodies neither divide (as they do in *P. dryomyiae*), nor unite (as in *P. hiemalis*), but that they develop directly by expansion into two subequal paranuclear masses.

If there is any significance in the fact that the first polar body divides before forming a paranuclear mass, or that the two polar bodies coalesce, or that they develop directly into paranuclear masses, it is not yet understood. In any event, the object accomplished is identical: paranuclear masses originate from the polar bodies, and are confined thereafter to a differentiated plasm of the egg known as the trophamnion. The function of the trophamnion is nutritive. It elaborates as the parasite body grows, and portions of it eventually surround each embryo of a polyembryonal mass, thus permitting each embryo to be so nursed that it can develop into a young larva. Henceforth the insect is able to provide for itself by direct feeding upon the host.

In other polyembryonic hymenopters Leiby (3) and the writers (4) have shown that the egg, before cleavage or very shortly thereafter, must be encompassed by host tissue in order to continue development. Eggs which are not so provided



cease development and become aborted. The egg of *Platygaster vernalis* is not encysted by host tissue, but is able to continue its development by reason of its location in the chyle of the host's stomach. The chyle, it should be recalled, is in reality the sap of the wheat plant, although it may be altered chemically by the nuclei of the intestinal lining; so that in one sense the parasite body may be regarded as developing in host tissue.

#### DEVELOPMENT OF THE PARASITE BODY IN THE HOST LARVA

The development of the embryos of *Platygaster vernalis* covers a period of about thirty days and takes place largely during the month of May, while the host larva is feeding and maturing upon the wheat plant. The presence of the parasite body in the stomach of the host apparently does not affect its growth. Like other species of polyembryonic parasites the *P. vernalis* larvæ are not developed to the point where they begin feeding upon the host until after the host is completely grown. Precocious development of the parasite larvæ, and feeding before the host is fully grown, would result either in their starvation or a reduction in the number produced from a single egg caused by the feeding of some of the parasites upon others of the brood.

The pupal and adult stages of the parasites are developed in individual cocoons (Pl. 1, E) formed by the larvæ in the carcass of the host during late summer. The adults remain in the cocoons during the winter and emerge the following spring.

#### EARLY CLEAVAGE AND THE FORMATION OF THE POLYGERM

About the second day after oviposition the cleavage nucleus migrates toward the center of the egg, where it divides to form two daughter embryonic nuclei (Pl. 3, C) of equal size. A second, third, and fourth cleavage of the embryonic nuclei take place between the third and sixth days after oviposition. These divisions of the nuclei produce parasite bodies which contain four, eight, and sixteen embryonic nuclei respectively (Pl. 3, D-F; Pl. 4, A). The embryonic nuclei are located in the central part of the parasite body in an enlarged embryonic region, which was differentiated around the cleavage nucleus when the parasite body was one day old. They are all of about the same size and measure approximately  $4\ \mu$  in diameter.

While the first four cleavages take place, the trophamnion becomes elaborated proportionately. The earliest change is observed at the first cleavage, when one of the paranuclear masses divides to form two masses which immediately proceed to increase in size (Pl. 3, C). At this time the parasite body measures  $24.3\ \mu$  in length and  $10\ \mu$  in width. At the second cleavage four paranuclear masses (Pl. 3, D) are observed in the trophamnion. At the third cleavage there may be as many as twelve paranuclear masses. As is shown in Plate 3, E, the masses are distributed uniformly throughout the trophamnion. The parasite body now measures approximately  $27.3\ \mu$  in length and  $14.5\ \mu$  in width. Its form is still ovoid, and similar to that of the original egg.

The development of *Platygaster vernalis* reaches that stage which is comparable to the polygerm stage of other polyembryonic hymenoptera at the end of the fourth cleavage (Pl. 4, A), at which time the parasite body contains sixteen nuclei in the embryonic region if all nuclei have divided regularly. Each embryonic nucleus then becomes separated from the others, and, surrounded by a bit of the embryonic cytoplasm, the cytoplasm with its nucleus becomes invested by a membrane. A cell is thus formed and recognized as a germ. The germ becomes the progenitor of one or two parasites, depending upon whether or not it divides once in the morula stage. The parasite body now represents a typical polygerm.

If all the nuclei divided regularly at the fourth cleavage the polygerm should contain sixteen germs. If only seven of the eight embryonic nuclei divided at the fourth cleavage, the polygerm would contain only fifteen germs. Difficulties of interpretation as to whether each germ is originally composed of a single cleavage nucleus are often encountered by reason of the fact that the single nucleus of a germ divides to form a binucleated germ before others of the same polygerm divide. Moreover, the nuclei are often so disposed (one above the other) and the germ membrane is sometimes so indistinct, that it appears as if some germs might originally be composed of two embryonic or cleavage nuclei.

The contention that a germ originates from a single embryonic nucleus in this polyembryonic species is based upon a study of such preparations as are illustrated by Plate 4, A and B. Figure A shows that most of the nuclei are each surrounded by a portion of the embryonic cytoplasm, which is in turn encompassed by a membrane. Figure B represents a later stage, and shows two germs containing but a single nucleus each, while two other germs contain two nuclei each as a result of a recent division of the original nucleus of each of the two germs. A fifth germ contains four nuclei, while a sixth has already advanced to the morula stage.

The polygerm is either slightly ovoid or spherical at this time and measures approximately  $47\ \mu$  in its greatest diameter. The trophamnion now is no longer confined to the periphery of a central embryonic region but penetrates toward the center of the polygerm so that it surrounds each germ.

About the twelfth day the polygerm (Pl. 4, C) measures about  $57\ \mu$  in diameter. With the increase in size of the polygerm there is also noted an increase in the size of each germ. At this stage certain of the germs divide in toto to form two daughter germs. Not all of the germs divide, and when division takes place the parent germ is usually composed of approximately eight nuclei. A similar division has been recorded by Marchal (5) in *Polygnotus minutus* and by the senior writer (3) in *Copidosoma gelechia*. A typical case of germ division is illustrated in Plate 4, C. Here two of the four germs are daughter germs that are still in contact, and separated only by a very thin portion of the trophamnion which filtered between them immediately after division. Henceforth the embryos develop rapidly into the blastula stage.

#### THE POLYBLASTULA STAGE

About thirteen days after oviposition the parasite body (Pl. 4, E) represents a typical polyblastula stage. The germs have increased both in size and in number of their nuclei. The nuclei become lodged in cells and are arranged regularly in the periphery of the germ, so that a median section through an embryo illustrates a true blastula. At the thirteenth day the polyblastula measures about  $55\ \mu$  in its greatest length and  $45\ \mu$  in width.

Between the thirteenth and eighteenth days the polyblastula increases in size until it measures about  $75\ \mu$  by  $68\ \mu$  (Pl. 4, F; Pl. 5, A). The oval or spherical shape of the parasite body is maintained. During this interval the blastulas and the cavities in which they are found increase in size proportionately, as do the paranuclear masses of the trophamnion. The trophamnion also appears somewhat vacuolated.

By the twentieth day the polyblastula measures when spherical about  $114\ \mu$  in diameter. At about this stage one observes definitely that all of the embryos are not approximately of the same size. Some of the embryos are normal blastulas, while others are composed of but four to ten nuclei and are in reality in the germ stage. Instead of the cavities in which the germs are located measuring  $40\ \mu$  in diameter, as do those of the typical blastulas of this age, they measure

only 18  $\mu$  in diameter. Two such germs are represented in the polyblastula illustrated in Plate 5, B. These germs have failed to develop, and will degenerate. They are similar to the pseudogerms described by the writers (3, 4) in *Copidosoma gelechiae* and in *Platygaster hiemalis*. Pseudogerms apparently do not occur in all *P. vernalis* parasite bodies. The writers believe that they are developed as a result of total division of a daughter germ, and that the resulting components do not contain sufficient potential elements to allow them to mature.

At about the twenty-fourth day the polyblastula is usually elongate. The one represented by Plate 6, A, measures approximately 0.4 mm. in length and 0.07 mm. in width. It contains seven blastulas which are about ready to begin organogeny of the embryo. In this particular parasite body the paranuclear masses are found at each end. In the less elongate but more ovoid parasite bodies the paranuclear masses continue to be distributed regularly throughout the trophamnion.

#### THE POLYEMBRYONAL MASS

Between the twenty-sixth and thirty-second days after the egg has been deposited the parasite body represents a typical polyembryonal mass. During this period organogeny of the embryos takes place. The parasite body is usually found intact in one end of the mid-intestine of the host at this time (Pl. 7, B), although occasionally it may become split up into two or three secondary polyembryonal masses.

A twenty-seven day old parasite body is illustrated in Plate 6, B. This parasite body measures approximately 0.52 by 0.19 mm. The embryos continue to be held together by the trophamnion, which is less dense than in the preceding stages. The paranuclear masses have increased in size, but these, too, are less concentrated.

The organogeny of the embryos, as far as has been determined in a general way, takes place similarly to that described by Marchal (5, 6) for *Polygnotus minutus* and other *Platygaster*s and by Silvestri (9) for *Platygaster dryomyiae*; and it will therefore not be referred to in detail. The blastulas become somewhat oval (Pl. 6, A) before the embryonic layers are differentiated. Prior to the formation of the mesenteron the embryos assume the U-shape (Pl. 6, B) described for other polyembryonic Hymenoptera, and then straighten out to form the primary larval stage (Pl. 7, B). During organogeny the embryos increase in size from 0.09 mm. in length, when they are somewhat U-shaped, to approximately 0.14 mm., when they resemble typical primary larvæ.

The final stage of the polyembryonal mass is illustrated in Plate 7, B, which represents a section through a parasite body at about the thirty-second day. The parasite body measures 0.8 by 0.4 mm. The embryos are in reality fully formed primary larvæ which have not yet taken any food, and measure approximately 0.23 by 0.07 mm. The fixed host in which this polyembryonal mass is found measures 3.8 mm. in length, while its mid-intestine measures 2.1 by 0.47 mm.

About the thirty-fourth day the membranelike trophamnion is ruptured by the first feeding of the primary larvæ, whereupon the larvæ are set free in the mid-intestine of the host (Pl. 1, D).

#### SUMMARY AND DISCUSSION OF CLEAVAGE TO LARVAL STAGES

It has been demonstrated above that the egg of *Platygaster vernalis* is organized into a typical polygerm, and finally into a polyembryonal mass, during the course of its later development, and before the newly formed primary larvæ are set free. This development takes place entirely in the mid-intestine of the

host, the parasite body being tossed about within it by peristaltic action, at least until the polygerm stage is formed. Thereafter, the polygerm becomes, as a rule, lodged in one end of the mid-intestine, where the germs develop into true embryos, and finally into the primary larval stage before the parasite body is broken up.

The development of *Platygaster vernalis* is therefore similar, in a general way, to that of *Polygnotus minutus*. In other described polyembryonic species, such as *Copidosoma gelechiae* and *C. truncatellum*, development takes place in the body cavity of the host, the polygerm breaking up into secondary masses which are held together in a group by adipose tissue of the host in the case of *C. gelechiae*, and scattered throughout the body of the host in the case of *C. truncatellum*. Each individual blastula or embryo of *C. gelechiae* and *C. truncatellum* becomes separated from all others and is invested with a portion of the trophamnion and paranucleus, thus completing its development independently of any other individual. In *Platygaster vernalis* and *Polygnotus minutus* the parasites are developed to the primary larval stage in the common original trophamnion, although, as mentioned above, the parasite body may, in a few instances, at least in *P. vernalis*, become accidentally divided into two or three secondary masses. In this event one of these secondary masses may not develop completely.

Before cleavage the egg contains a cleavage or embryonic nucleus in a differentiated embryonic region, the remainder of the egg comprising the trophamnion, which contains two paranuclear masses of polar body origin. Four cleavages of the original embryonic nucleus result in the production of twelve to sixteen daughter embryonic nuclei, each of which lies within a small portion of the embryonic plasm and forms a germ. Some of the germs when composed of eight embryonic nuclei divide to form two daughter germs, but in any event each normal healthy germ finally develops into an embryo. From eight to twelve embryos are thus developed from a single *Platygaster vernalis* egg.

The development of the *Platygaster vernalis* egg therefore represents a simple type of polyembryony; not as simple as that shown by the writers (4) in *P. hiemalis*, nor as complex as that demonstrated in other polyembryonic insects by Marchal (5), Silvestri (7), and Leiby (3). In *P. hiemalis* we have shown that immediately after second cleavage some of the eggs divided into two equal parts, each part containing two embryonic nuclei and two paranuclear masses which together form an embryo. Twin embryos are thus developed from some eggs, while other eggs do not become so organized, and develop but a single individual. In *P. vernalis* approximately four cleavages take place before the germs are formed and an average of eight embryos are thus matured from a single egg. In *Copidosoma gelechiae* the senior writer (3) has shown that the germs are not organized until after the seventh cleavage, with the result that from 150 to 225 embryos are produced from a single egg. In any event, it appears that the number of embryos produced nearly always approximates the number of parasites that the host larva is able to mature. The size of the host appears to be the governing factor. Where but one egg is normally deposited in the host egg, as in the case of *C. gelechiae* and *P. vernalis*, cleavage continues to the point where the maximum number of parasites is developed that the host can mature. When cleavage extends beyond that number the embryos become aborted in their different stages. In the case of *P. hiemalis* two individuals develop from some of its eggs, and a single individual from others. This parasite therefore deposits from five to eight eggs at a time in one host egg, and in this way are developed the maximum number of individuals of this species that the Hessian fly larva can mature.

The failure of some of the germs to keep pace in their development with other germs in the same host has been briefly referred to above; the fault being ascribed

to cleavage of daughter germs. A similar condition obtains for some of the blastulas, but the instances are rare. Occasionally a group of three or four blastulas will fail to continue development. Examination of the parasite body shows in such instances that a portion of it has become separated in the host intestine from the rest of the parasite body, and that the detached portion did not happen to become provided with a sufficient amount of the trophamnion and paranuclear masses. Such blastulas then become aborted.

Occasionally host larvæ are met with which contain more than one parasite body, in spite of the tendency of the parasite to oviposit in an egg only once, and to deposit at that time only a single egg. At such times one of the parasite eggs may fail to continue development. An undeveloped parasite body of this kind is illustrated in Plate 4, D.

Aborted eggs, germs, blastulas, or larvæ in *Platygaster vernalis* are rare in comparison to those which have been observed in *Copidosoma gelechiae* and in *C. truncatellum*, a condition which is to be expected in polyembryonic insects which show a comparatively simple type of polyembryonic development.

### THE LARVA

As has been shown by the junior writer (1, 2) the larva passes through two distinct stages in completing its development. When the insect takes its first food it is known as the primary larva (Pl. 8, A). In this stage the larva is elongate oval, bluntly rounded at both ends, and possesses two relatively large mandibles. This stage is further characterized by a lack of distinct body segmentation, and the presence of two very prominent lateral knoblike projections which are located in the head region at the base of the mandibles. The primary larva measures about 0.54 mm. in length and 0.18 mm. in width.

The mature larva (Pl. 8, B, C) is white, ovoid, and measures about 1 mm. in length and 0.5 mm. in width. Spiracles are present in this stage on the second and third thoracic segments, and second abdominal segment only. The mandibles of the mature larva are less than half the length of those of the primary larva. Eleven distinct body segments are defined in the mature larva.

Feeding commences when the primary larva is fully formed. The larvæ of a brood first consume the remnants of the trophamnion, whereupon they become liberated in the mid-intestine of the host (Pl. 1, D) and ingest the chyle. The intestine is next ruptured and the fatty tissues consumed. In the course of feeding, the entire contents of the host larva are consumed, leaving only the outer cuticle (Pl. 8, C) to contain the fully developed parasite larvæ. In the process of feeding, the superior lip is moved toward and away from the inferior lip by radiating muscles which are quite prominent in the head region.

A parasitized larva very seldom succeeds in pupating, but it does form the puparium. Each parasite larva, when fully grown, forms a cocoon, so that a brood of the parasites in the larval or pupal stages is contained within a cluster of cocoons (Pl. 1, E), which is in turn confined in the puparium of the host (Pl. 1, F).

### THE PUPA

The pupa (Pl. 8, D) is formed in the cocoon. At the time of transformation it is white in color, but gradually the eyes and body darken until it is a shiny black. The parasites spend two to three weeks in the pupal stage.

### THE ADULT

The adult is shiny black and measures from 0.7 to 0.9 mm. in length. An average of about eight individuals, which are nearly always of the same sex, are reared from one host. After emergence in spring they will live in confinement

from three to twenty-nine days, the length of time depending upon the food and the humidity of the atmosphere.

Oviposition by fertilized or unfertilized females takes place immediately after emergence if the parasite happens to come in contact with a host egg. Experiments conducted by the junior writer (2) show a definite tendency for a female to oviposit only once in a host egg. The preparations, studied by the writers, which resulted from ovipositions controlled in the laboratory, indicate conclusively that a single egg is deposited at each oviposition.

#### SEX RATIO

A study of the sex of individuals of the broods indicates that usually all of a brood are either males or all females. Of 48 broods, 40 were either pure male or pure female broods, and 8 were mixed. A similar ratio has been shown by Marchal (5) for *Polygnotus minutus*; in the article cited he records the sex of 16 broods; eight being pure female broods, six male broods, and two mixed. Marchal believes that a mixed brood originates as a result of a fertilized and an unfertilized egg being deposited in the host. The writers believe that a similar explanation will answer for *Platygaster vernalis*.

#### SUMMARY

(1) *Platygaster vernalis* develops polyembryonically in the larva of the Hessian fly, one egg giving rise eventually to approximately eight individuals. There is but one generation annually.

(2) The adult parasites emerge from their cocoons in spring and almost immediately oviposit in the eggs of the host. By the first of June the embryos are fully formed in a well-grown host larva. The larvæ feed upon the host during June and July, and then transform to pupæ, which in turn become adults in August.

(3) A single egg is deposited by the parasite at each oviposition, and in such a manner that the egg always becomes lodged in the host's mid-intestine, where development to the larval stage is completed. Development begins immediately, whether the egg is fertilized or unfertilized.

(4) If more than one egg is deposited in the same host by different females one of the eggs may become aborted.

(5) In the course of maturation two polar bodies are formed, which become the two original paranuclear masses. The matured oöcyte or cleavage nucleus becomes the progenitor of the embryos.

(6) Four divisions of either the conjugated or parthenogenetic cleavage nucleus result in the production of twelve to sixteen embryonic nuclei, each of which apparently gives rise to a germ. The germs develop in the central part of the parasite body and are encompassed by the trophamnion containing paranuclear masses.

(7) Some of the germs divide once, at the time they are composed of eight nuclei, to form two daughter germs. A further division of the daughter germs apparently results in the production of pseudogerms.

(8) The group of germs comprising a parasite body is known as a polygerm. Each normal germ passes through the blastula and late embryonic stages, and finally becomes a primary larva. During the course of this development the parasite body increases in size and remains intact in the mid-intestine of the host.

(9) When the primary larvæ are formed they rupture the thin trophamniotic membrane and begin to feed upon the contents of the host's mid-intestine. Later the mid-intestine is ruptured, whereupon the secondary or mature larvæ devour the entire contents of the host, leaving only the cuticula.

(10) Each larva constructs a cocoon in which it transforms to a pupa and later an adult parasite. The cluster of cocoons is surrounded by the cuticula of the host, and is further protected during the winter by the host's puparium.

(11) The adults of a brood are usually of the same sex. It is believed that the occasional mixed broods originate from a fertilized and an unfertilized egg deposited in the same host egg.

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PLATE 1

*Platygaster vernalis*

A.—Female parasite poised on a blade of wheat and ovipositing in egg of Hessian fly. ×45.

B.—Egg of *P. vernalis* before oviposition. Much enlarged.

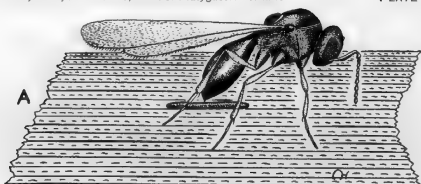
C.—Longitudinal section through a young Hessian fly larva, showing a *P. vernalis* parasite body in the mid-intestine. ×55.

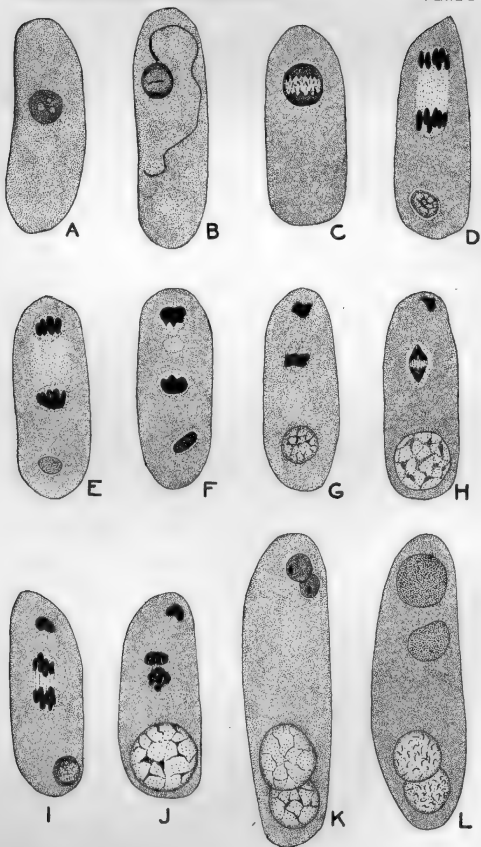
D.—Longitudinal section through a well grown Hessian fly larva showing primary stage larvae of *P. vernalis* in the mid-intestine. ×40.

E.—Host larval carcass (cuticula) containing ten *P. vernalis* cocoons. ×15.

F.—Host puparium showing exit holes made by adult parasites. ×14.







## PLATE 2

### *Platygaster vernalis*

All figures drawn 2,200 times natural size

A.—Unfertilized egg, immediately after oviposition, containing the nucleus.

B.—Fertilized egg 30 minutes after oviposition, with nucleus and vermiform sperm.

C.—Unfertilized egg five hours old, with the nucleus beginning the first maturation.

D.—Fertilized egg six hours old, showing the male nucleus and the first maturation spindle.

E.—Like D, but at slightly later stage. The anterior nucleus becomes the first polar body, the middle nucleus the oöcyte nucleus of the second order.

F.—Like D and E, but slightly further advanced.

G.—Fertilized egg 10 hours after oviposition. The first polar body and the oöcyte nucleus of the second order show their nuclear material much condensed, while the male nucleus has meanwhile expanded and become spherical.

H.—The beginning of second maturation of a fertilized egg, about 12 hours after oviposition.

I.—Like H, but a slightly later stage.

J.—Egg 12 hours old. Second maturation is completed, and a second polar body and the female pronucleus are formed.

K.—Between the twelfth and twenty-fourth hours the egg increases slightly in size. The two polar bodies have migrated to the anterior end of the egg, and begin to increase in size.

L.—Polar bodies become elaborated and are henceforth known as paranuclear masses. Male and female pronuclei about to unite. Egg 24 hours old.

### PLATE 3

#### *Platygaster vernalis*

All figures drawn 2,200 times natural size

A.—Portion of fertilized egg 24 hours old with male and female pronuclei at prophase.

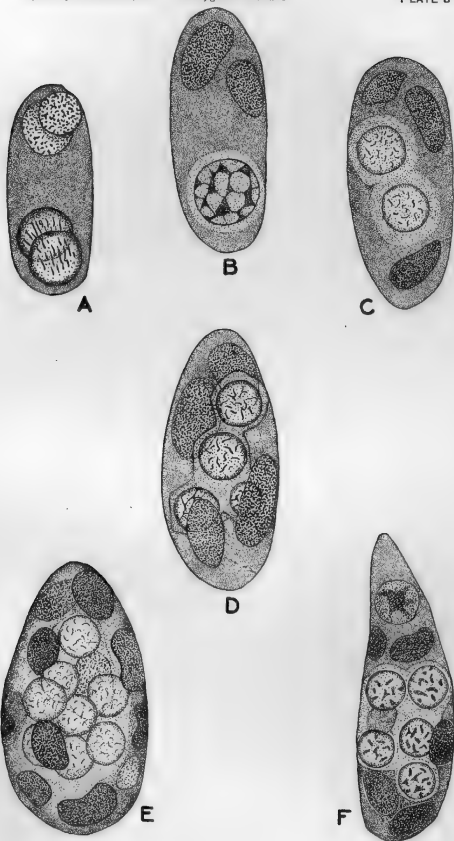
B.—Fertilized egg one to two days after oviposition, with the cleavage nucleus in a differentiated embryonic region. An unfertilized egg of this age has a similar appearance, except that the cleavage nucleus is not quite so large.

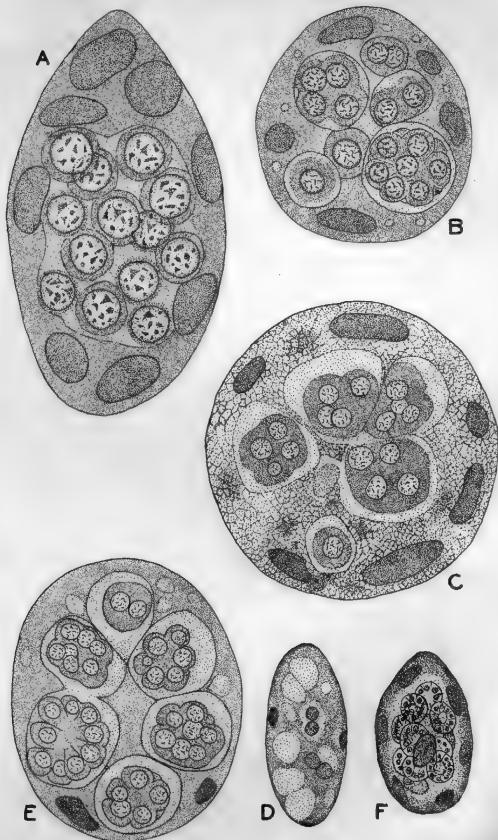
C.—Egg (now the parasite body) two to three days old. One of the paranuclear masses has divided amitotically. First division of the cleavage nucleus has produced two cleavage nuclei in a differentiated embryonic region.

D.—Section through a three-day old parasite body, showing four cleavage or embryonic nuclei and four paranuclear masses.

E.—Drawing of entire parasite body four days old. The eight embryonic nuclei are located in a central or embryonic region, which in turn is surrounded by the polar region now known as the trophamnion containing paranuclear masses.

F.—Section of a parasite body about six days old. Five of the eight embryonic nuclei are shown.





## PLATE 4

### *Platygaster vernalis*

A.—Section of parasite body about seven days old, showing thirteen of its sixteen embryonic nuclei forming germs. The parasite body has increased in size considerably.  $\times 2200$ .

B.—Section through a polygerm about ten days old, showing five germs and one morula. The darker nuclei in the periphery are paranuclear masses.  $\times 1100$ .

C.—Section through a polygerm about twelve days old, showing portions of five germs. The two upper germs have just been produced by the division of a parent germ. Each germ is lodged in an embryonic cavity which is surrounded by a somewhat vacuolated trophamnion containing paranuclear masses. Since development takes place in the mid-intestine of the host, no cyst of host tissue ever surrounds the parasite body.  $\times 1100$ .

D.—Aborted or pseudoparasite body, found sometimes in host with a healthy parasite body, in the process of degenerating. Twelve days after oviposition.  $\times 1100$ .

E.—Section of a polygerm about thirteen days after oviposition, showing portions of six embryos in the germ or early blastula stage, and two paranuclear masses in the trophamnion.  $\times 1100$ .

F.—In toto drawing of a polyblastula with nine blastulas in the embryonic region which is surrounded by the trophamnion, containing its dark staining paranuclear masses.  $\times 550$ .

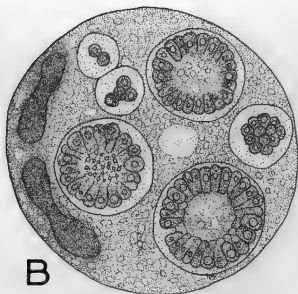
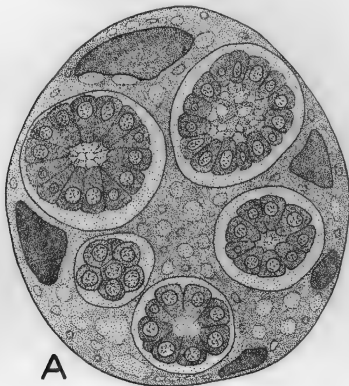
PLATE 5

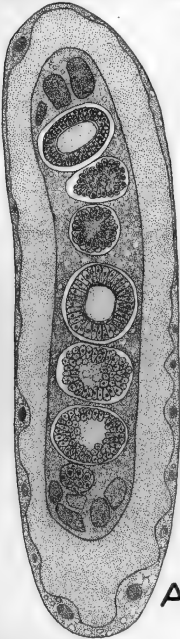
*Platygaster vernalis*

A.—Section through an 18-day-old parasite in the polyblastula stage showing the embryos at the early blastula stage. The trophamnion becomes noticeably vacuolated and surrounds the embryonic cavities on all sides.  $\times 1100$ .

B.—Section through a 20-day-old parasite body, showing four normal embryos in the blastula stage, two paranuclear masses and two pseudogerms which are apparently degenerating because division of the parent germ was carried too far.  $\times 550$ .

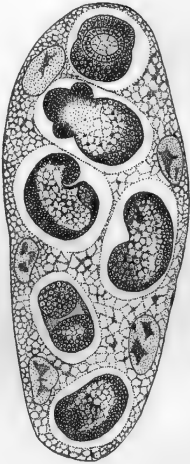






A

Journal of Agricultural Research



B

Washington, D. C.

## PLATE 6

### *Platygaster vernalis*

A.—Section of a polyblastula 23 days old located in the chyle of the mid-intestine of the host. Portions of seven of the eight blastulas which compose this parasite body are shown. Two of the blastulas are cut through the center. This parasite body has assumed an elongate shape and now measures about one-fourth the length of the mid-intestine of the host. The paranuclear masses are shown at each end. The periphery of the drawing represents the epithelium of the mid-intestine, the lighter stippled area the chyle of the mid-intestine.  $\times 275$ .

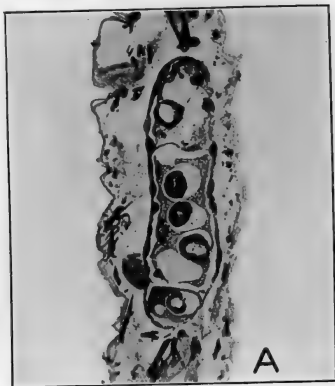
B.—Longitudinal section through a polyembryonal mass about 26 days after the original egg was deposited, showing portions of six embryos. About this time the embryos are forming the germ layers and organs of the young larvae and are somewhat Gothic-like-shaped. Note that the trophamnion is less dense, and that the paranuclear masses are no longer so conspicuous.  $\times 200$ .

## PLATE 7

### *Platygaster vernalis*

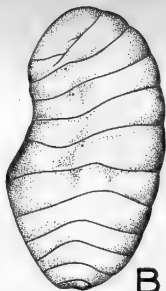
A.—Photomicrograph of polyembryonal mass distributed throughout the mid-intestine of the host. Sections of five embryos are shown which have just differentiated the germ layers. Note that the trophamnion is vacuolated.  $\times 93$ .

B.—Photomicrograph of polyembryonal mass lodged in the anterior end of the host's mid-intestine. The embryos have almost or quite reached the stage of the primary larva. The black area surrounding the embryonal mass represents the chyle of the host. The lower primary larva is just beginning to devour the remnants of the trophamnion.  $\times 62$ .

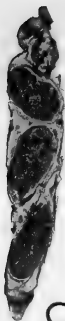




A



B



C



D

PLATE 8

*Platygaster vernalis*

- A.—Primary larva.   ×111.
- B.—Mature larva.   ×62.
- C.—Photomicrograph of section through a host larva (carcass), showing three *P. vernalis* larvæ that are almost mature.   ×25.
- D.—Pupa of *P. vernalis*.   ×62.

# THE INHERITANCE OF PUBESCENT NODES IN A CROSS BETWEEN TWO VARIETIES OF WHEAT<sup>1</sup>

By H. H. LOVE, *Department of Plant Breeding, Cornell University Agricultural Experiment Station*, and W. T. CRAIG, *Agent, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture*

Nearly all varieties of wheat have glabrous nodes, but there are some which have pubescent (velvet) nodes. One of these was furnished to the authors by J. A. Clark, of the Office of Cereal Investigations. It was a bearded variety with glabrous glumes, which had been found by Ball and Clark as a rather common admixture in a plat of mixed Pacific Bluestem and Baart (Early Baart) wheat on the Adams County Branch Station at Lind, Washington. It was given Cereal Investigations accession number 5877 and, tentatively, the name "Velvetnode." As it does not occur as a commercial variety, it does not appear in the published classification of American wheats. Its most peculiar character was the possession of hairy nodes.

To study the inheritance of the hairy node the Velvetnode variety was crossed with a strain of unknown origin in the plant-breeding nursery. It was being carried under the name "New Columbia" but is not related to the variety grown commercially under that name, which is the same as Fultzo-Mediterranean. The Cornell strain is beardless, has pubescent glumes, and glabrous nodes. This strain has been given Cereal Investigations No. 5946. It is most closely related to Mealy but has very purple stems.

The first generation of this cross resulted in plants that were beardless, with pubescent glumes and nodes. The plants seemed to be perfectly fertile and the heads were well filled with seed.

The seeds from these F<sub>1</sub> plants were sown in the field in the fall of 1921, and the resulting plants were studied during the following summer. Although it had been thought that the Velvetnode was a spring variety, all of the plants seemed to survive the winter and produced fairly thrifty plants.

At harvest time the plants were pulled and sorted first for pubescent nodes only. While there was some variation in the length and number of hairs on the nodes there was no attempt to distinguish between the different types, and all individuals that had any hairs on the node were classed as pubescent. The segregation seemed to indicate a 3:1 ratio with pubescent node dominant. On observing the two lots of plants, however, it was noted that all of the bearded plants were in the group with hairy nodes. No bearded plants were found in the glabrous-noded class. The plants were then reclassified according to the characters of nodes and beards. The results of this segregation are shown in Table I.

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<sup>1</sup> Received for publication April 16, 1924. Paper No. 130, Department of Plant Breeding, Cornell University, Ithaca, New York. These results were obtained in investigations conducted cooperatively by the Cornell University Agricultural Experiment Station and the Bureau of Plant Industry, U. S. Dept. of Agriculture.



TABLE I.—Showing the segregation in  $F_2$  for beards and pubescent nodes

	Pubescent node		Glabrous node	
	Beardless	Bearded	Beardless	Bearded
Observed.....	468	202	237	0
Calculated 2 : 1 : 1.....	453.5	226.75	226.75	0
Deviation.....	14.5	24.75	10.25	0

These results show that there were no bearded plants with glabrous nodes. The presence of beards usually is due to one factor for beards and the ratio obtained usually is 3 beardless to 1 bearded, and the pubescent-node character occurs in the simple 3 : 1 ratio with pubescence dominant, as described above. Therefore, when the grouping is made according to the two characters one would expect to obtain some plants that were bearded with glabrous nodes unless linkage occurs. In this case the data seem to indicate that there may be a linkage between beards and pubescent nodes. These two characters come in together and out of 907 plants there is not a single case of a crossover type. This also may suggest that beards and hairy nodes result from one factor and that the difference between the parent forms is due to one factor pair. For the present such an assumption will be made.

In respect to the node character the Velvetnode variety may be designated by  $VV$  and the beardless strain with glabrous nodes by  $vv$ . The  $F_1$  gametes may then be represented by  $Vv$ . In  $F_2$  there will be the following types and in the ratio indicated:—1  $VV$  : 2  $Vv$  : 1  $vv$ . The numbers obtained deviated somewhat from the calculated numbers and the value of  $P$  is 0.168, which does not indicate a very good fit. When the different plants are tested in  $F_3$ , however, it seems that the facts support this 1 : 2 : 1 assumption. There are certain exceptions that will be mentioned later.

According to this assumption, the bearded plants with pubescent nodes ( $VV$ ) all will breed true; the same is true also for the beardless plants with glabrous nodes ( $vv$ ). The beardless hairy-noded plants ( $Vv$ ) all should segregate again into the three types.

Of the  $F_2$  plants, 238 were chosen for testing in the  $F_3$ . The numbers from the different classes were as follows: 123 beardless with pubescent nodes, 68 bearded with pubescent nodes, 47 beardless with glabrous nodes.

From the  $F_2$  generation, 123 plants that were beardless with pubescent nodes were tested in  $F_3$ . According to the above assumption these would be expected to segregate into beardless glabrous, beardless pubescent, and bearded pubescent, in the ratio of 1 : 2 : 1. Of these plants, 117 segregated in this manner, and, while the number of individuals is not large in some of the families, yet the total number of plants is rather large. The total number is 1,295 beardless glabrous, 2,524 beardless pubescent, and 1,158 bearded pubescent. The calculated numbers for the three classes are 1,244.25 : 2,488.50 : 1,244.25.

There is considerable difference between the calculated and observed numbers. Part of this may be due to the fact that some plants became very ripe in the field before they were harvested (all the notes were taken in the field), and owing to the shattering of some of the awns or beards it was difficult at times to be sure a plant was bearded. The value of  $P$  in this case is only about 0.014, which shows a poor fit for a case of this sort. If it were not for the breeding behavior, one would conclude that the deviation from expectancy was too great to substantiate the hypothesis assumed.

In addition to these 117 plants there were certain ones that showed a different behavior. For example, there were four  $F_2$  beardless plants with pubescent nodes whose progeny did not segregate into the three groups as did those of the 117 plants noted above. Two of these plants produced plants with hairy nodes only, but segregated for beards. These produced 76 plants that were beardless with pubescent nodes and 20 plants that were bearded with pubescent nodes. While these numbers are not large, nevertheless in other cases with smaller numbers the expected 1:2:1 ratio was obtained or approached. The other two  $F_2$  plants produced only beardless plants in  $F_3$ , but segregated for pubescent and glabrous nodes.

Thus, out of 123 plants of the  $F_2$ , 117 reproduced the three types in the expected ratio, 1:2:1, that would follow providing the difference between the parent forms is due to one pair of factors. The occurrence of these six plants that did not give the expected ratio suggests that there may be more than one pair of factors concerned.

From the  $F_2$  generation 68 plants that were bearded and had pubescent nodes were selected to test in  $F_3$ . All of the 68 plants bred true to the type selected; that is, all plants produced were bearded with hairy nodes.

There also were 47 beardless plants with glabrous nodes selected from the  $F_2$  generation to test in  $F_3$ . All but two of these plants produced beardless plants with glabrous nodes.

The possibility that more than one pair of factors is concerned is further emphasized by the fact that there were two  $F_2$  plants classed as beardless with glabrous nodes that did not breed true to this character, as would be expected, but segregated for beards. These produced 38 beardless plants with glabrous nodes and 19 bearded plants with glabrous nodes. While in the  $F_2$  no bearded plants with glabrous nodes were obtained, yet in  $F_3$  it is seen that some plants of this class do occur occasionally.

In addition to the plants whose behavior in  $F_3$  is cited above, there were five that produced some bearded plants with glabrous nodes, but the numbers were so small that it may be questioned whether they may not be accidental crosses or mixtures of some kind. These plants are being tested further.

The six  $F_2$  plants that have been mentioned as giving ratios differing from the expected ratios may be crossovers; and, if so, the genetic constitution assumed would have to be modified. A very close linkage would be indicated and the difference between the parent forms would be considered as due to two pairs of factors rather than one. It may be possible that these six aberrant plants result from natural hybridization in the field. This, however, is rather unlikely as no simple cross will explain certain of the results. For example, take the case of the two plants that produced all beardless plants in  $F_3$ , but segregated for pubescent and glabrous nodes. As a 3:1 ratio for node type was obtained, it is difficult to see how this could arise from hybridization. If true-breeding pubescent-noded beardless types existed, this could be explained by a cross with a beardless glabrous-noded type, but so far no such constant type has been found.

It does not seem wise to conclude that natural crossing may be the cause of these few plants which produce different ratios from the majority of plants found in the same  $F_2$  classes.

It is possible that they may be explained by mutations occurring in the germ cells, but at present no attempt will be made to assign a definite cause. After further breeding tests, now under way, are finished, a definite explanation can be given.

The plants of  $F_2$  and  $F_3$  also were classified for pubescent glume to determine whether this character segregated independently. The distribution of the  $F_2$

plants for the three characters concerned is shown in Table II, together with the data for 55 F<sub>2</sub> plants tested in F<sub>3</sub>. From the results of both F<sub>2</sub> and F<sub>3</sub> it is evident that the pubescent-glume character segregates independently and is not linked in any way so far as this material is concerned. Considering pubescent glume alone, it segregates in a simple 3:1 ratio with pubescence dominant.

TABLE II.—*Showing the distribution of the F<sub>2</sub> and F<sub>3</sub> plants according to beards, pubescent nodes, and pubescent glumes*

	Beardless				Bearded			
	Pubescent glume		Glabrous glume		Pubescent glume		Glabrous glume	
	Pubescent node	Glabrous node	Pubescent node	Glabrous node	Pubescent node	Glabrous node	Pubescent node	Glabrous node
Results from F <sub>2</sub> .....	344	184	124	53	153	0	49	0
Results from 55 F <sub>2</sub> plants in F <sub>3</sub> .....	963	475	355	192	448	0	139	0

CONCLUSION

The results presented indicate that the character pubescent node as found in the Velvetnode variety is very closely linked with the bearded condition. Whether the few aberrant cases are really crossovers or may be explained in another way can not be definitely established at this time. The breeding tests that are now under way will shed more light on this question.



# JOURNAL OF AGRICULTURAL RESEARCH

## CONTENTS

	Page
Density of Cell Sap in Relation to Environmental Conditions in the Wasatch Mountains of Utah - - - - - C. F. KORSTIAN	845
An Ascigerous Stage and Synonymy for <i>Fusarium moniliforme</i> - - - - - GRACE O. WINELAND	909
Expulsion of Aecidiospores by the Mayapple Rust, <i>Puccinia podophylli</i> Schw. - - - B. O. DODGE	923
The Heat of Wetting of Soil Colloids - - - - - M. S. ANDERSON	927
Comparison of Pima Cotton with Upland Varieties in Arizona - - - - - C. J. KING, H. F. LOOMIS, and D. L. VARMETTE	937
The Fungus Causing the Common Brown Rot of Fruits in America - - - - - JOHN W. ROBERTS and JOHN C. DUNEGAN	955
Preliminary Results with the Borax Treatment of Citrus Fruits for the Prevention of Blue Mold Rot - - - - - HARRY R. FULTON and JOHN J. BOWMAN	961
Snails as Predisposing Agents of Sugar Cane "Root Disease" in Louisiana - - - R. D. RANDS	969
Greenhouse Experiments with Atmospheric Nitrogen Fertilizers and Related Compounds - - - - - F. E. ALLISON, E. B. VLIET, J. J. SKINNER, and F. R. REID	971

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# JOURNAL OF AGRICULTURAL RESEARCH

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## DENSITY OF CELL SAP IN RELATION TO ENVIRONMENTAL CONDITIONS IN THE WASATCH MOUNTAINS OF UTAH<sup>1</sup>

By C. F. KORSTIAN

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### INTRODUCTION

Observers of plant life in the mountainous regions of the West are familiar with the striking zonation of the vegetation in series of altitudinal belts of distinctive composition. These range from sagebrush deserts or grasslands at the base of the mountains through successive altitudinal forms representing such diverse vegetative aspects as pinon-juniper woodlands, pine forests, aspen stands, Douglas fir, and, on the summits, subalpine spruce-fir forests. Except for the sagebrush and greasewood-shadscale associations, each zone is practically co-extensive with a forest type.<sup>2</sup>

The phenomenon of altitudinal zonation is of particular significance to the silviculturist and the range management specialist because of the fact that many of the problems of the growth and regeneration of forests and the development of the range can best be solved through a determination of the soil and climatic requirements of the different species of each zone. The relation of environmental conditions to the development of forest vegetation is thus of profound importance. For example, in designating the kinds and amounts of timber which can be cut from an area without prejudicing the future composition or rendering the forest liable to windthrow, the alert forester is continually making use of his fund of knowledge of the physiological activities and requirements of the various species in his region. In reforestation activities, success depends upon knowing the cause of successful growth and establishment or of partial success or failure of different species in various forest types under varied environmental conditions. Once the problems of growth and regeneration of forests have been solved, the forester should be able to determine readily what species

<sup>1</sup> Received for publication Feb. 28, 1924—issued Nov., 1924. In the following pages authors are cited for the most important and the more recent researches. References are not given for material which is regarded as a matter of common knowledge among scientists. To those who wish more complete citations for the period up to the time of their publication: Ewart's excellent translation of Pfeffer's "Physiology of Plants" (102), Livingston's "Rôle of Diffusion and Osmotic Pressure in Plants" (83), and Dixon's "Transpiration and the Ascent of Sap in Plants" (33) will be found of great service. In order to give the background for the work and to correlate the present work with that of previous workers in the subject, references to the more important literature are given in connection with the topical discussions.

<sup>2</sup> "Forest type" may be defined as a forest of essentially uniform character as regards composition and development due to given physical and biological factors by which it may be differentiated from other forests of unlike composition. It is evident, therefore, that the environmental conditions responsible for a given forest type must have been practically the same throughout the type at the time the forest was started and during its development. A forest type is usually designated by the name of the dominant tree species which determine the leading characteristics of the type.

will attain the best growth and development on a given aspect at a given elevation. When the effects of climatic or other factors are once definitely known, failures in planting may be largely avoided by a judicious choice of sites<sup>3</sup> and of especially adapted species.

Since reliable quantitative data on the physiological requirements of native forest vegetation are still very meager, investigations were undertaken to determine site characteristics by studies of native plants as indicators. (72, 73.)<sup>4</sup>

The physiological activities of a plant are, of course, a response to (and therefore must be a measure of) the physical factors of the environment. By numerous close observations it ought, therefore, to be possible to ascertain the exact conditions required for optimum growth and development. If this could be done quantitatively, by the employment of native species as indicators, a way might be found to determine with precision the planting sites most suitable for species with known requirements. In the studies which were accordingly undertaken in the Intermountain Region, which is situated mainly in the Great Basin and the Snake River drainage, it was early found that the water relations of the plant and its environment are of paramount importance, especially where semiarid conditions prevail<sup>5</sup> (76, 77). It is very evident that no environmental factors are more significant than the forces with which the soil withholds and the air withdraws water from the plant.

The results of a study of one phase of the water-relations problem are reported in this paper, namely, the relation which the density of the cell sap bears to environmental conditions in the Wasatch Mountains of Utah.<sup>6</sup> The importance of this relationship will be brought out more fully further on. Before taking up the details of the present investigation, however, the mechanism of absorption, diffusion, and osmotic pressure in plants will be considered briefly as the basis for a proper understanding and interpretation of what follows.

## RELATION BETWEEN OSMOTIC PHENOMENA AND CELL SAP CONCENTRATION

Because of its restricting cell walls a plant is compelled to depend entirely upon fluid nutriment. The absorption of water and of dissolved substances by the root hairs and young rootlets is practically the only natural method by which the plant can obtain its supply of nutrients from the soil. The passage of water through the organism, however, is not a simple process. In the first place, not all water-soluble substances, as shown by Jost (69, p. 11-23), are able to enter the cell; secondly, to reach the interior of the cell, the fluid, with its solutes, must penetrate both the cell-wall and the protoplast—the layer of living protoplasm within the cell. The passage of water through the organism is therefore complicated by the presence of substances in solution, and this involves osmosis, or the

<sup>3</sup> "Site" is regarded by foresters as the summation or combined effect of the climatic and edaphic conditions of an area or environment when considered with reference to its forest-producing power; and is practically synonymous with "habitat" as defined by the ecologist. The relation of the living tree to its inert environment embraces the most fundamental of silvical problems. In fact, a great many of the physiological problems of plant life involve to a greater or less extent the relation of the organism to its environment. The plant in relation to its environment must be regarded, therefore, as a physiologically active organism. The existence, limits, and movements of plant communities are controlled by the physical conditions of the environment.

<sup>4</sup> Reference is made by number (italic) to "Literature cited," pp. 900-907.

<sup>5</sup> This is further elaborated in a report by F. S. Baker and C. F. Korstian now in process of preparation, covering climate, soil, and native vegetation of brushlands as indicators of planting sites for western yellow pine in the intermountain region.

<sup>6</sup> Since the sap upon which the determinations were based was extracted by pressure from the tissues a part is doubtless intercellular rather than intracellular in origin. This is probably but a very small fraction of the whole amount, and can introduce no appreciable error in the results.

movement of the solvent through the cell-wall membranes from the weaker to the stronger solution.

The physicochemical laws of diffusion and osmosis, which underlie the water relations of plants, can only be roughly outlined in this paper. For more complete discussions of these laws, relating particularly to selective absorption, diffusion, and osmotic pressure, the reader is referred to the literature cited herewith, and to standard works on physical chemistry and plant physiology. Many investigations have been conducted to determine the influence upon the organism of the medium in which it is grown, but it is only recently that the osmotic pressure of the cell sap has been studied in this connection. Among such researches those of Drabble and Drabble (37), Fitting (40), Dixon and Atkins (26-31, 34-36), Harris, Gortner and Lawrence (46, 48-50, 52-61), Iljin, Nazarova and Ostrovskaja (68), and Livingston (86, 87) should be mentioned.

The foundations of our present knowledge of osmotic phenomena were laid by Pfeffer (101) in 1877 and somewhat later were further amplified by Van't Hoff (67). LeClerc du Sablon (79) and Livingston (83) have shown that the permeability of membranes is an essential factor in the physiology of plants and that all of the material exchanges, whether between the cells themselves or between the cells and the external medium (soil in the case of forest trees), are determined by the laws of osmosis.

These laws set forth explicitly that a substance in solution tends to distribute itself uniformly throughout the entire volume of the solvent. In the plant cell no absorption will take place when the concentration of the solution outside is equal to that within the cell vacuole. If the outside solution is the more highly concentrated, water will actually be withdrawn from the vacuole, which will become smaller. An osmotic interchange through the cell walls thus accompanies and complicates the simple movement of water, and though millions of cells may intervene there exists a direct osmotic relation between the topmost leaf of the tallest Sequoia tree and the soil solution at its root tips.

Osmotic pressure can not be measured directly except with great difficulty, but is readily calculable from the amount by which a solute lowers the freezing point or raises the boiling point of the solvent or from the observed vapor tension of a solution. These indirect methods depend upon the general principles that the depression of the freezing point, elevation of the boiling point, decrease of the vapor tension of solutions, and osmotic pressure are all related phenomena and may be obtained one from the other for any given solution.

A review of the above-mentioned researches convinced the writer that an investigation of the osmotic properties of the intra-cellular fluids of forest vegetation on various sites, selected with reference to wide differences in evaporation and the supply of available soil moisture, would throw considerable light upon the basic physiological problems of silviculture and forest distribution. Chief among these may be mentioned the relative ability of different species to extract water from the soil, and, to some extent, retard transpiration through increased sap concentration; the relation of sap density to frost resistance; and the rôle of the osmotic pressure of the cell sap in the adaptation of exotic species introduced on dry sites in semiarid regions.

## EXPERIMENTAL METHODS<sup>7</sup>

In conducting this investigation it was necessary to consider: (1) the selection of sites, (2) the collection of leaf material, and (3) the technique of determining the density of the cell sap.

<sup>7</sup> The writer desires to express his indebtedness to Doctors Harris and Gortner for further details of technique gained through a short sojourn at their field laboratory and through their subsequent visit to the writer's field laboratory in 1920.



## SELECTION OF SITES

If differences in the density of the sap are in any way correlated with site, they will presumably be the more pronounced the more diverse the site conditions. Extremely varied site conditions were available in Big Cottonwood Canyon, southeast of Salt Lake City, Utah. The Cottonwood Nursery of the Forest Service proved a suitable place for the establishment of a properly equipped base from which to carry on the tests. Material was collected all the way from the margins of streams in the sagebrush desert southeast of Salt Lake City to timberline on the summit of the Wasatch Range. A supplemental collection was also made on the Beaver Creek watershed near the western end of the Uinta Mountains in connection with other work. Two winter collections were made on the Taylor Canyon watershed in the Wasatch Mountains east of Ogden, Utah. These were not limited wholly to the most typical portions of the sites which are discussed below, but were made as opportunity offered and subsequently classified with reference to site. The differences between the sites, as shown by the averages in the tables which follow, might at first appear not as large as they should be, but it must be remembered that one site grades imperceptibly into another; and the transition between areas as well as the typical portions of each site should, therefore, furnish their quota of determinations. As a further control on this series of tests another was made on the Ephraim Canyon watershed extending from the alkali flats on the desert west of Ephraim, Utah, to the summit of the Wasatch Plateau, where detailed climatic records have been secured from 1913 to 1922 at three different elevations.<sup>8</sup> Many of the samples for these tests were collected in the immediate vicinity of the climatic stations, thus offering an opportunity for correlating sap density with climatological data.

## COLLECTION OF LEAF MATERIAL

The leaves were gathered as quickly as possible, and were packed tightly in thick-walled test tubes of about 100 cc. capacity. Every precaution was taken to prevent contamination. Except when additional material was being put in, the tubes were kept tightly closed with rubber stoppers, to preclude loss of water by evaporation and consequent increase of the sap concentration. When material was collected which was still wet with rain or dew the leaves were at once wiped dry with some clean absorbent substance. Before placing the test tubes in the freezing mixture the possibility of contamination was further precluded by capping them with oiled paper held firmly by a strong rubber band. As soon as possible after collection the tubes were placed in a slushy mixture of finely chopped ice and salt, where a temperature of  $-15^{\circ}$  to  $-17^{\circ}$  C. was maintained. They were later thawed out for the extraction of the sap. Although any suitable receptacle would serve for the freezing mixture, a double-walled water-tight wooden freezing box holding three to four dozen test tubes at a time proved the most satisfactory and efficient equipment for the purpose. Each of the two compartments of the freezing box was provided with a false top which had 18 to 24 holes in it for holding the tubes firmly with their upper ends just above the freezing mixture. The box was covered with a lid which projected down over the sides for about 4 inches. With this type of portable freezing box it was possible to make two-day trips by automobile to remote points and return to the field laboratory with the samples still frozen.

<sup>8</sup> The writer is under particular obligation to Dr. A. W. Sampson, formerly plant ecologist, and H. E. Malmsten, grazing examiner, Great Basin Grazing Experiment Station, who aided very materially in the conduct of the control tests through actual participation in the work, and who have kindly placed summaries of the climatological records at the writer's disposal in advance of their publication in a forthcoming bulletin of this department. Inasmuch as the detailed records are soon to be published by these investigators, the writer has made use of the summaries only, the most of which are shown graphically in figure 5.

## TECHNIQUE OF DETERMINATION OF DENSITY OF SAP

The importance of freezing the samples preliminary to extraction of the sap has been fully discussed by Dixon and Atkins (29), by Gortner and Harris (41), and by Gortner, Lawrence, and Harris (42). These studies indicate that this preliminary freezing is essential to increase the permeability of the tissues which yield a more representative sample of the solutes from the smaller as well as from the larger vacuoles. Sap from unfrozen tissues has in all cases resulted in lower concentrations than that from frozen tissue, because of the less complete extractions of the former.

Sap density determinations were made by means of the standard freezing-point depression method used by Cavara (16) as early as 1902-1905, later by Dixon and Atkins (29), and further developed by Harris, Gortner, and Lawrence (41, 42, 46, 47), whereby the osmotic pressure of the sap is calculated from the observed depression of the freezing point. Freezing points were determined by means of the classical Beckmann thermometric method (6), which has been widely used in chemistry and biology. The thermometer was read to thousandths of degrees and the results were then expressed in freezing point lowering in degrees C. ( $\Delta$ ) and in atmospheres of osmotic pressure (P), after corrections for undercooling were made by using the formula suggested by Harris and Gortner, as follows:

$\Delta = \Delta' - 0.0125 \mu \Delta$ , in which  $\Delta$  = the true depression of the freezing point,  $\Delta'$  = the observed depression of the freezing point,  $\mu$  = the amount of undercooling in degrees C., and a constant (0.0125) experimentally determined, according to the tables calculated by Harris and Gortner. As a part of the laboratory routine it was necessary to determine the freezing point of double distilled water at the beginning and the end of each day's work. Through the adoption of the simplified technique it became possible to make 50 freezing point determinations in one day, all of which were made by the writer.

On several occasions during the course of these investigations the importance was forcibly brought out of exercising extreme precautions in the collecting and freezing of the samples and the extraction of the sap. Otherwise there can be no assurance that the results obtained will be dependable. Evaporation, especially, must be most carefully watched. A striking example of this is afforded in the case of *Orthocarpus tolmiei*. Specimens of this species collected in an opening in the spruce-fir forest in Big Cottonwood Canyon on September 9, 1920, at an elevation of 8,850 feet and treated in the regular manner showed a freezing point depression of the sap of 1.76° C. or an osmotic pressure of 21.2 atmospheres. In marked contrast, the sap of leaves collected from the same plants at the same time but allowed to lie in the shade exposed to the air for 12 hours depressed the freezing point 2.67° (32.0 atmospheres). This difference was caused largely by the drying out of the leaves. With the water supply cut off and the evaporation of moisture from the stomata continuing, a material concentration of the remaining sap is the obvious result.

Another striking example occurred in connection with two collections of conifers in the spruce-fir type of Ephraim Canyon on July 18 and 22. Both lots were collected and frozen in accordance with the prescribed methods, except that one lot on each date was allowed to stand subjected to hot summer temperatures on the laboratory table for six hours after it had been taken from the freezing mixture but before being taken out of the freezing tubes, while the sap of the other lot was extracted and tested immediately after the leaves had thawed out. The results of this comparison are given in Table I.

TABLE I.—Effect on sap density of exposure to room temperature

Species, aspect, and collection date	Depression of freezing point in degrees C.		Osmotic pressure in atmospheres	
	Normal treatment	Left standing 6 hours at room temperature	Normal treatment	Left standing 6 hours at room temperature
<i>Picea engelmanni</i>				
Northern aspect:				
July 18.....	1.37	1.62	16.5	19.5
July 22.....	1.17	2.01	14.1	24.2
Southern aspect:				
July 18.....	1.16	2.38	14.0	28.6
July 22.....	1.15	1.74	13.8	20.9
<i>Abies lasiocarpa</i>				
Northern aspect, July 22.....	0.78	1.85	9.4	22.2
Southern aspect:				
July 18.....	1.36	1.57	16.4	18.9
July 22.....	0.98	1.66	11.8	20.0
<i>Pinus flexilis</i>				
Southern aspect:				
July 18.....	1.20	1.92	14.4	23.1
July 22.....	1.14	1.95	13.7	23.4

It is obvious that the higher concentration in the sap from the tubes which were allowed to stand for 6 hours on the laboratory table was not due to evaporation, since the stoppers had not even been removed after the samples were collected.

The fact that the sap which was expressed from these leaves showed a darker color and had a very unusual odor, slightly rancid and somewhat alcoholic, strongly suggests that oxidation and enzymic action had already set in and that the sap density had thereby been materially changed. These results also suggest the grave danger of using any method of extracting the sap which provides for soaking the leaves in warm water, as this treatment creates conditions which are very conducive to enzymic activity.

When the leaf tissues were thoroughly frozen the tubes were removed from the freezing mixture, washed under a warm water tap to remove salt from the surface and by thawing the tissues quickly to enable a more complete plasmolysis. The tubes were carefully wiped on the outside and around the mouth to remove any remaining salt which may have adhered. The tissues were then removed from the tube and folded in a small square of very heavy muslin which had been previously boiled in two changes of distilled water and carefully dried. The sap was then extracted by thorough and complete pressing in a press specially designed for this purpose.<sup>9</sup> Several different types of presses were employed but most of them failed to exert the pressure required to extract sap from many of the plants growing on the semiarid sites and from the conifers having a mucilaginous sap. The press which finally proved fully satisfactory consisted of a heavily tinned press bowl turned from a 4-inch piece of shaft steel, having a convex surface rising from the inside of the bottom. A plunger made of similar material hollowed out at the end was, by means of the screw in a heavy pipe vise, forced down on the tissue lying between the convex surface of the bowl and the concave surface of the plunger.

The screw was turned down slightly every few minutes to maintain a constant oozing of the sap from the tissue. The tissue was frequently rearranged and

<sup>9</sup> The writer is under particular obligation to Dr. J. Arthur Harris for material assistance and cooperation in this connection.

pressure applied again until as much as possible of the sap was expressed. It was found essential to subject all samples to the same uniform thorough pressing because the last sap obtained from a sample is more concentrated than that which is obtained at first.<sup>10</sup>

### ENVIRONMENTAL CONDITIONS

The salient physiographic and geologic features of the central Wasatch region have been well described by Butler and Loughlin (15). It is an area of strong relief, extending from an elevation of approximately 5,000 feet at the western base of the range to altitudes of almost 11,500 feet on the highest summits. Many of the slopes are steep and include areas of bare, polished rock. The canyons are U-shaped, the branch canyons being of the "hanging" type and the heads of canyons have the basin or cirque form characteristic of recent glaciation. The important rocks of the region consist of quartz diorite, Cambrian limestone, quartzite and shale which decompose into sandy to gravelly loam or silt loam soils.

The topography of the Wasatch Plateau in the vicinity of Ephraim Canyon is much less rugged than that farther north, due mainly to the fact that the sedimentary sandstones and limestones interbedded with geological clay comprise the greater part of the geological formations. The top of the mountain range is often quite flat and a mile or more in width. The soil is, for the most part, a heavy, highly calcareous clay loam or clay.

The salient vegetational features of the main Rocky Mountain region have been very well described by a number of writers, among whom may be mentioned Bates (3, 4), Pearson (99), Ramaley (104, 105), Robbins (108), Rydberg (110), and particularly Sampson (113, 114), who has studied extensively the ecological relations of the vegetation of the Wasatch Mountains. There are, however, some major vegetational differences between the Wasatch Mountains and the central and southern Rocky Mountains. Pinon pine (*Pinus edulis*) drops out of the pinon-juniper type in north central Utah, leaving Utah juniper (*Juniperus utahensis*) the dominant species of the type. Western yellow pine (*Pinus ponderosa scopulorum*) is found very infrequently in the northern part of the Wasatch Range occurring either upon the limited areas of sandy or gravelly soils or near streams where soil moisture conditions are exceptionally good. In Big Cottonwood Canyon western yellow pine occurs naturally on only one site—a gravelly talus slope having a steep southern aspect.

Another striking contrast between the Wasatch Mountains and the Rockies lies in the greater abundance of aspen (*Populus tremuloides*) in the Utah aspen-fir type, where it frequently forms pure stands on old burns. This temporary type of aspen may be considered a retrogression in the succession of forest types due to fire. Lodgepole pine (*Pinus contorta*) is less prominent in Utah, disappearing entirely south of the headwaters of the Provo River on the Uinta National Forest.

Considerable variation in composition is apparent within the same type in different localities, evidently due principally to factors in the environment and to disturbances in the natural succession. For example, a pure stand of Douglas fir, after being burned over, may be followed by a more or less temporary stand of aspen—a retrogression to a sub-climax stage in the developmental succession. However, the aspen will be gradually and eventually wholly supplanted by the Douglas fir. In Taylor Canyon at present there is little aspen on the potential aspen-fir sites, while it is abundant in this type in Big Cottonwood Canyon. Another notable contrast between the forest types on these two watersheds is

<sup>10</sup> This has been reported also by Gortner, Lawrence, and Harris (42) and by Knudson and Ginsberg (71).

the absence of Engelmann spruce from the higher elevations on the Taylor Canyon watershed. Engelmann spruce is not found on the west slopes of the Wasatch Range immediately overlooking the large valley which was originally the Great Salt Lake Desert. Its absence is probably caused by the exposure of this side of the range to the full sweep of the prevailing southwesterly winds which blow across the desert and cause excessive evaporation both summer and winter. During the winter they also pile the snow in deep drifts on the leeward side, exposing the bare ground on the slopes to the windward and thus favoring winterkilling there.

In the Wasatch Mountains the study covered a range of 5,500 feet, from the bottom of the valley at an elevation of 4,500 feet where the sagebrush (*Artemisia tridentata*) is characteristic, through the juniper, chaparral, and aspen-fir types to the spruce-fir (*Picea engelmanni-Abies lasiocarpa*) type at 10,000 feet above sea level. The Wasatch Plateau series of observations covered a similar range in altitude from the greasewood-shadscale (*Sarcobatus vermiculatus-Atriplex* sp.) type at 5,500 feet, through the sagebrush, pinon-juniper, oakbrush, (*Quercus utahensis*) and aspen-fir to the spruce-fir type at 10,000 feet elevation. Each of the major plant associations is practically identical with definite vegetative types or life zones, such as those proposed by Merriam (93). Utah juniper is found from 4,500 to 7,000 feet in the Wasatch Mountains and the Rocky Mountain juniper (*Juniperus scopulorum*) from 6,000 to 8,500 feet (7,500–9,000 feet on the Wasatch Plateau). Douglas fir (*Pseudotsuga taxifolia*) occurs from 5,500 feet on northern aspects to 10,000 feet on southern aspects (7,500–9,000 feet on the Wasatch Plateau), while white fir (*Abies concolor*) is found from 5,500 feet on northern aspects to about 8,500 feet on southern aspects (7,500–9,000 feet on the Wasatch Plateau). Engelmann spruce occurs from 7,000 feet along streams to 10,000 feet, while alpine fir is found from 7,500 feet on northern aspects to 10,500 feet. On the Wasatch Plateau these two species range from about 8,500–11,000 feet. Blue spruce (*Picea parryana*) has a limited natural range extending from 7,000 to 8,000 feet along stream courses in the canyon bottoms (6,500–8,500 feet on the Wasatch Plateau). Limber pine (*Pinus flexilis*) occurs from 7,500 feet on northern aspects to timber line (10,500–11,000 feet). Aspen extends from about 6,800 feet in canyon bottoms up through the Douglas fir and spruce-fir types to an elevation of 10,000 feet. Narrowleaf cottonwood (*Populus angustifolia*) and other broadleaved species are found along stream courses from the lower valleys to about 7,500 feet.

TABLE II.—Comparison of mean maximum, mean minimum, and mean temperatures by months in degrees F. for Salt Lake City, and Cottonwood Nursery, Utah<sup>a</sup>

Stations and temperatures	January	February	March	April	May	June	July	August	September	October	November	December	Average, May-October	Annual
Salt Lake City (4,408 feet*above sea level; 45 years record):														
Mean maximum.....	36.6	41.0	50.9	59.9	68.2	79.1	87.9	86.6	76.2	62.9	49.7	39.2	76.8	61.5
Mean minimum.....	21.7	25.8	32.7	39.9	46.6	55.1	63.2	62.5	52.6	42.1	32.1	25.0	53.7	41.6
Mean.....	29.2	33.4	41.8	49.9	57.4	67.1	75.6	74.6	64.4	52.5	40.9	32.1	65.2	51.6
Cottonwood Nursery (7,450 feet above sea level):						1916 and to 1918	1916 to 1919	1915 to 1920	1915 to 1920	1915-17 and 1919-20				
Mean maximum.....						56.0	72.6	80.6	78.3	67.5	53.1		68.0	
Mean minimum.....						29.0	37.7	44.3	41.6	36.0	25.9		35.8	
Mean.....						42.5	55.2	62.4	60.0	51.8	39.5		51.9	

<sup>a</sup> Data furnished by J. Cecil Alter either in manuscript or published form (1, 2).

The usual relation between altitude and temperature holds in a general way in the Wasatch Mountains. Comparisons of temperatures for Salt Lake City and Cottonwood Nursery are shown in Table II. It is impossible from the mean monthly temperatures to form a clear idea of the relative duration of temperatures suitable or unsuitable for plant growth; that is, the temperatures above or below 40° F. Accordingly the average number of hours having temperatures above and below 40° F. and the number of hours of freezing temperature for June to September inclusive at three different elevations on the Ephraim Canyon watershed on the Manti National Forest about 100 miles south of the Big Cottonwood watershed for the period from 1913 to 1922, inclusive, were determined (Table III).

TABLE III.—*Summation of temperatures with reference to elevation on the Ephraim Canyon watershed*

Elevation (feet)	Type	Average number of hours June-September		
		Above 40° F.	Below 40° F.	Of freezing temperature
7,100.....	Oak brush.....	2, 786	142	39
8,700.....	Aspen-fir.....	2, 672	256	78
10,000.....	Spruce-fir.....	2, 499	426	126

Temperature gradients with altitude have been worked out by several investigators for different mountainous regions in the West. Pearson (99) finds a mean altitudinal temperature gradient of 3.68° F. for each 1,000 feet from an elevation of 3,300 feet at Kingman in northern Arizona to timber line on the San Francisco Mountains at 11,500 feet. Shreve (115) reports a gradient of 4.11° F. in the Santa Catalina Mountains of southern Arizona. Observations on other mountains in the West, according to Hann (43, p. 243-246), have resulted in the following gradients: Sierra Nevadas in Placer County, California, 4.12° F.; Pikes Peak, Colorado, 3.46° F. Air temperatures decrease quite uniformly with an increase in altitude, except for local inversions which are occasionally encountered due to air drainage in canyons and mountain valleys.

Precipitation in the Wasatch Mountains increases rapidly with altitude up to and including the aspen-fir type, as shown in Table IV. From this type to the spruce-fir type the increase is less pronounced, but the snowfall in the spruce-fir type on the high mountains is considerably heavier. Although not strictly comparable because the records for these stations do not cover the same period, they are nevertheless significant. They indicate an increase in the annual precipitation of 6.4 inches for each thousand feet. Alter's (1) study of the relation between precipitation and altitude, for practically all groups of adjacent stations with simultaneous records, on the western slope of the Wasatch Mountains, shows an increase in precipitation of from 15 inches annually at the lower elevation (4,250 feet) to 33 inches at the higher elevation (8,700 feet), or uniformly 4 inches per year per 1,000 feet. On the eastern slope on the leeward side of the range the precipitation decreases more rapidly with decrease in altitude, being nearly 5.5 inches for each thousand feet for the composite of all stations studied by Alter.

TABLE IV.—Comparison of mean monthly and annual precipitation in inches for various altitudes in the Wasatch Mountains<sup>a</sup>

Stations	January	February	March	April	May	June	July	August	September	October	November	December	Average precipitation May to October	Average annual precipitation
Salt Lake City (4,408 feet above sea level):														
Years of record, 1874-1920....	1.35	1.47	2.07	2.01	2.00	0.81	0.52	0.77	0.97	1.54	1.37	1.35	6.61	16.23
Lower Mill Creek (4,959 feet above sea level):														
Years of record, 1914-1920....	2.46	2.18	2.46	2.35	2.38	1.10	0.95	0.58	1.75	2.51	1.77	1.67	9.27	22.16
Cottonwood Nursery (7,450 feet above sea level) <sup>b</sup> .....					2.52	1.31	1.42	1.60	2.52	3.28			12.65	
Silver Lake (8,700 feet above sea level) <sup>c</sup> .....	5.47	6.41	6.28	4.39	2.56	0.48	1.05	2.03	3.17	3.84	2.93	5.24	13.13	43.85

<sup>a</sup> Data furnished by J. Cecil Alter either in manuscript or published form (1, 2).  
<sup>b</sup> Years of record are: May, 1916 and 1918; June, 1914-1919; July-October, 1914-1920.  
<sup>c</sup> Years of record are: January and February, 1907 and 1916-1922; March, 1916-1922; April and May, 1916-1921; June, 1909 and 1916-1921; July, 1907, 1908, and 1915-1921; August, 1907, 1909, and 1915-1921; September and October, 1907-1909, and 1915-1921; November and December, 1915-1921.

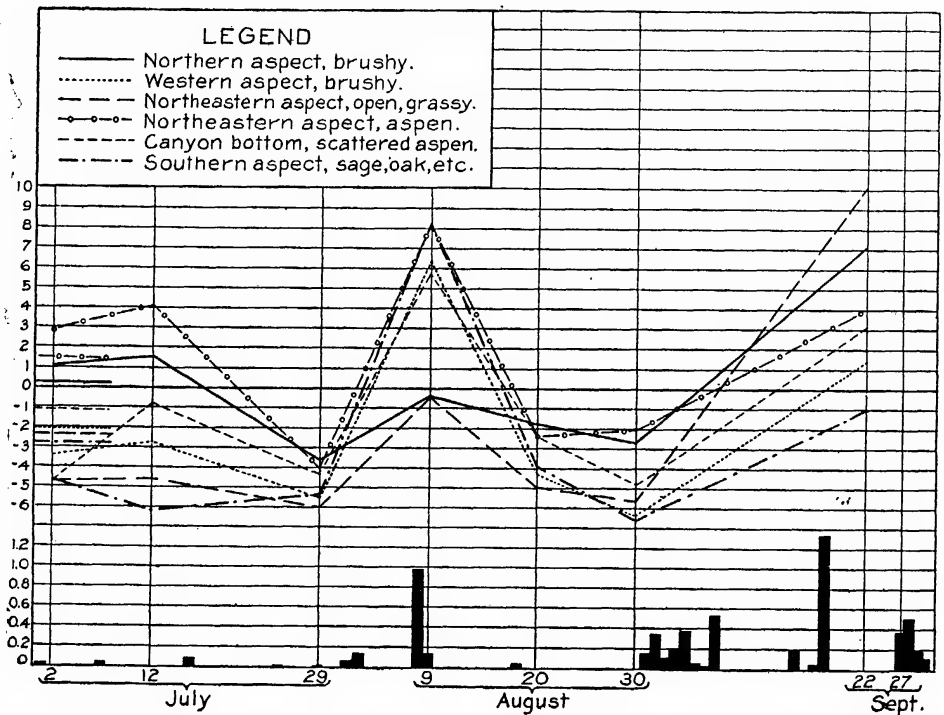
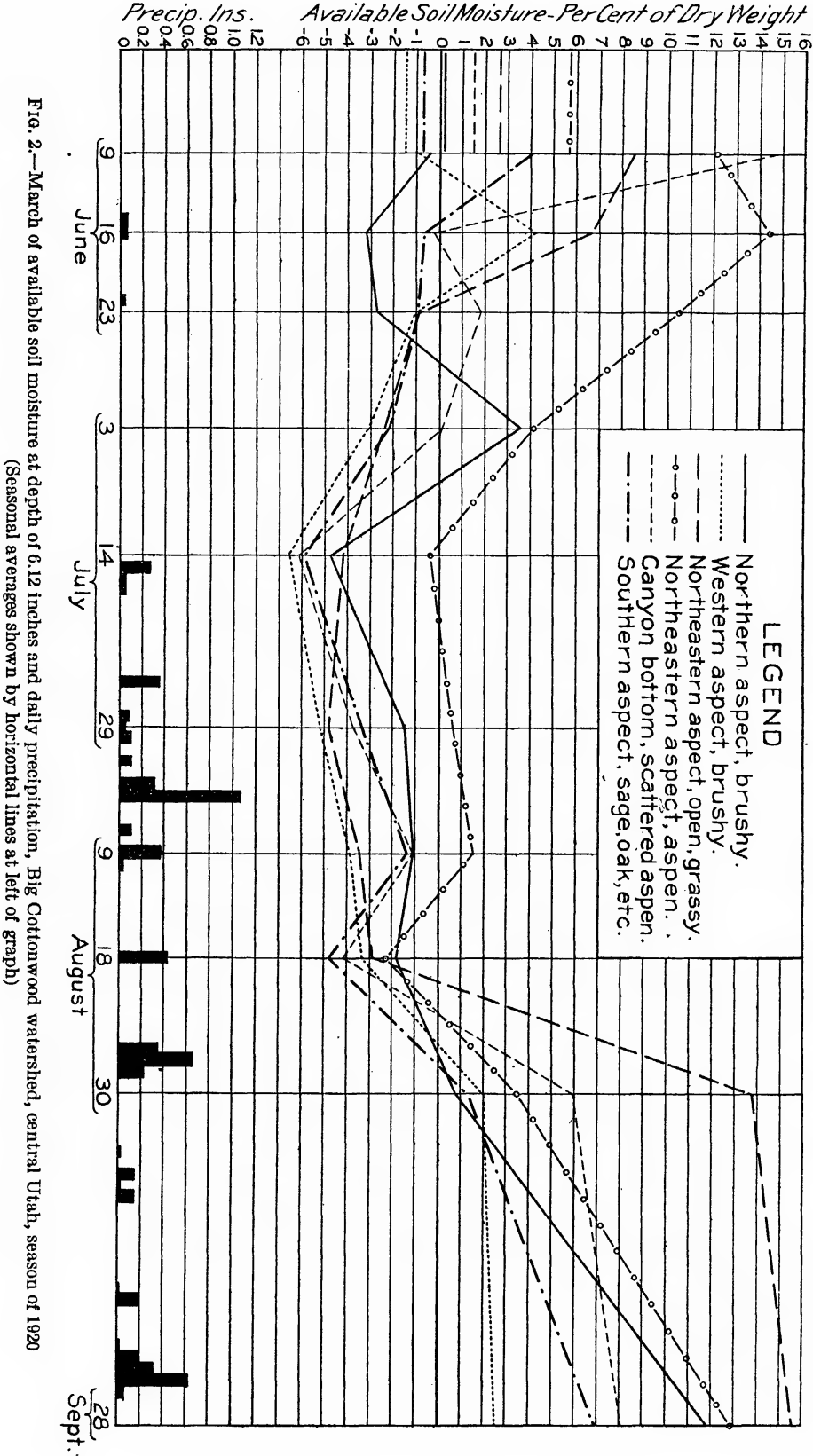


FIG. 1.—March of available soil moisture at depth of 6.12 inches and daily precipitation, Big Cottonwood watershed, central Utah, season of 1919  
 (Seasonal averages shown by horizontal lines at left of graph)

Very low summer precipitation and marked dryness of the surface soil characterize the Wasatch Mountain sites at low altitudes. At the Cottonwood Nursery June and July, with less than 1.5 inches, have the lowest average monthly precipitation. August has only 1.60 inches. The meagerness of the rainfall is further reflected in the amount of available soil moisture. The changes in soil moisture and in evaporation were recorded in 1919 and 1920 on several typical





sites in the aspen-fir zone. The data are presented in figures 1 and 2. The changes in the evaporation on these sites in 1919 and 1920 and in the soil temperature at a depth of 1 foot in 1920 are shown in figures 3 and 4.<sup>11</sup> A study of figures 1 and 2 and Tables V and VI shows that the surface foot of soil is frequently dried out for considerable periods below the limit of availability for plant growth. The soil moisture determinations, which were made in connection with studies of forest planting and were therefore confined to the upper foot of soil, indicate that on the more severe sites the vegetation must, for extended periods, draw the requisite amount of moisture from the lower layers of soil in which the plants are rooted. The upper stratum supplies soil moisture only intermittently following heavy rains which are of infrequent occurrence during the height of the growing season.

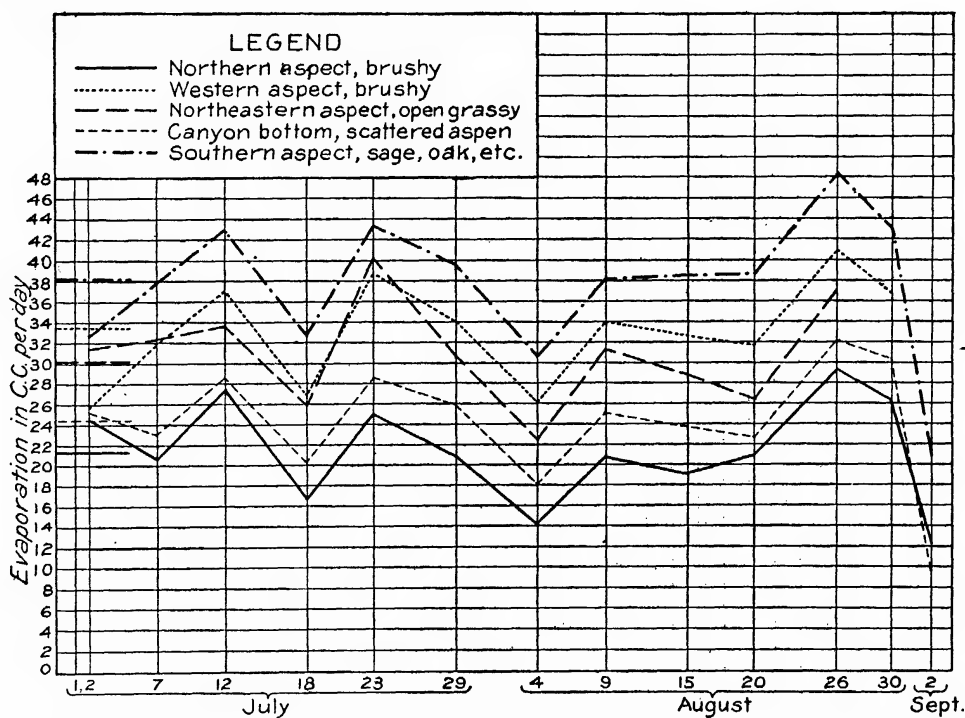
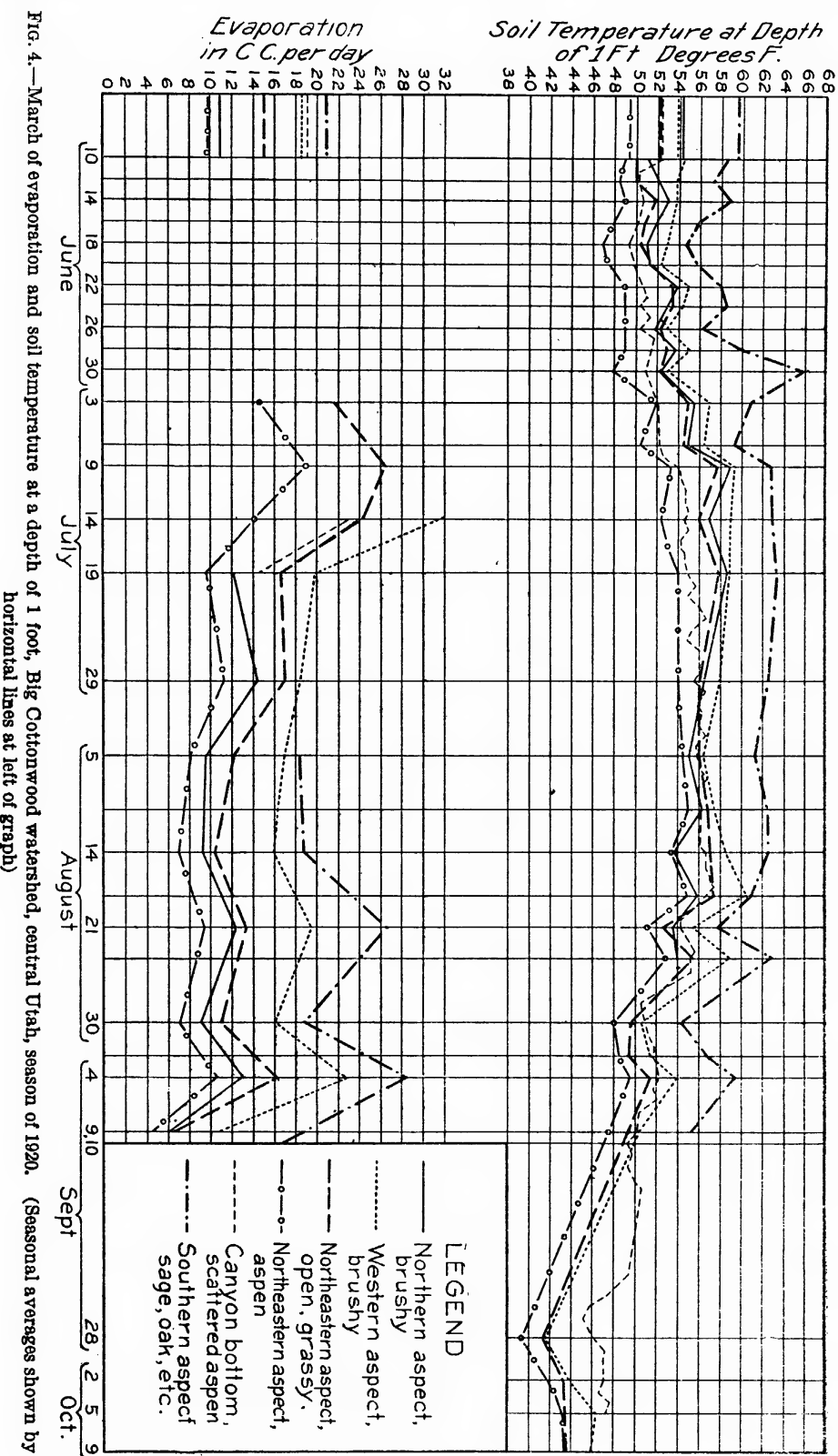


FIG. 3.—March of evaporation in Big Cottonwood watershed, central Utah, season of 1919  
(Seasonal averages shown by horizontal lines at left of graph)

The deficiency of summer precipitation at low altitudes is aggravated, from the standpoint of forest planting, by rapid evaporation, which shows a marked response to aspect. The mean daily evaporation rates are also shown for each site. In comparing the moisture relations of the different sites the evaporation-soil moisture ratio has been used. This ratio is the quotient of the evaporation divided by the mean seasonal moisture content of the soil (in this case a depth of 6–12 inches). A systematic gradation occurs from the more or less xerophytic southern aspect, through the four intervening sites, to the mesophytic northeastern aspect covered with a good stand of aspen. The aspen succeeded a forest of Douglas fir and white fir which was removed by logging and fire.

<sup>11</sup> The two years' record of evaporation was obtained by means of Shive's improved form of the Livingston standardized rain-correcting porous cup atmometer.



Precipitation on the Wasatch Plateau is notably less than at similar elevations in the Wasatch Mountains. The mean annual precipitation, based on averages of from five and one-half years in the oak-brush type to twenty-eight years' record at Manti in the desert valley about 6 miles from the mouth of Ephraim Canyon, is summarized by types in figure 5, together with the mean annual maximum and minimum temperatures, average number of hours above and below 40° F., and the average number of hours of freezing temperature per year.

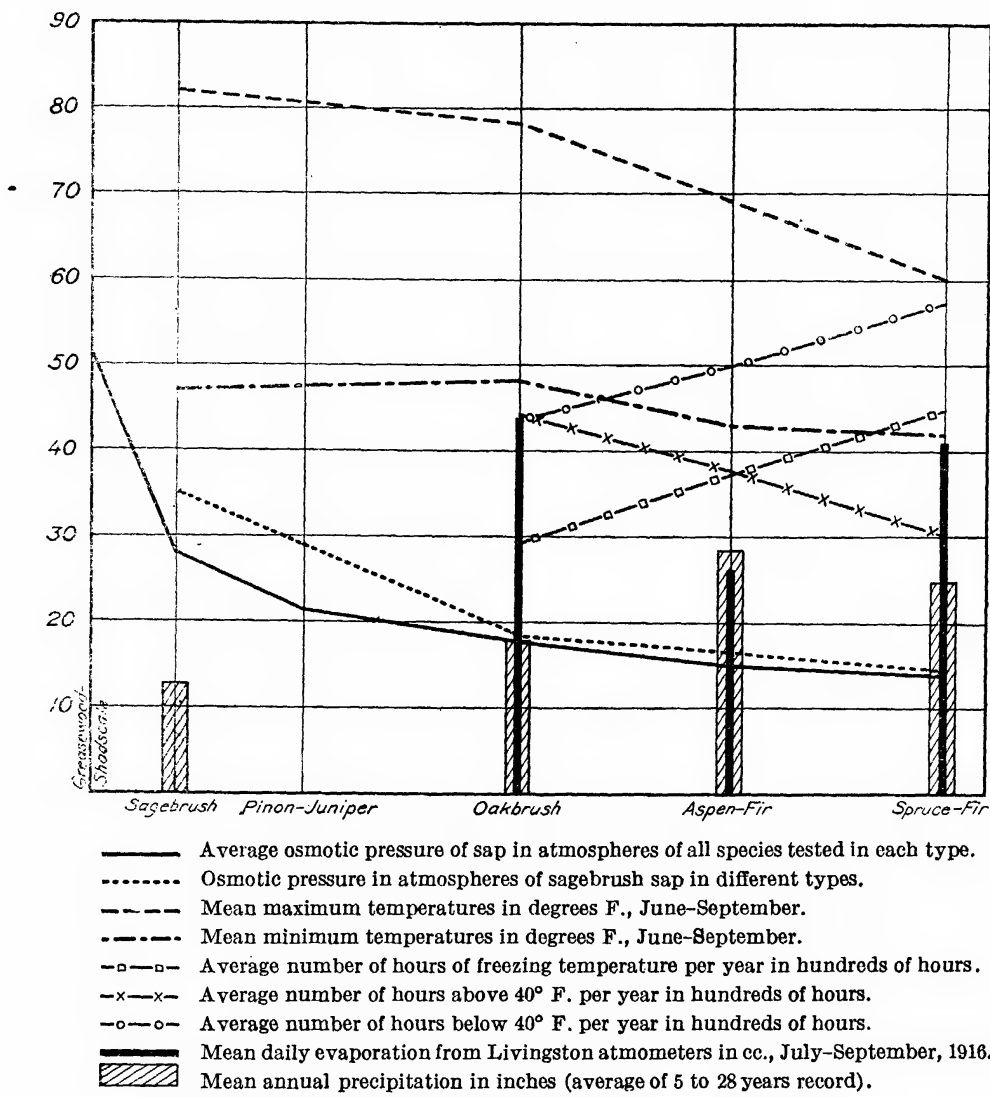


FIG. 5.—Summation of sap densities and principal climatic factors by types

Associated with an increase in altitude is a characteristic decrease in the mean maximum and mean minimum air temperatures, and in the average number of hours above 40° F. On the other hand, there is the usual increase with elevation both in the average number of hours below 40° F. and in the average number of hours of freezing temperature. The mean daily evaporation from July to September, inclusive (fig. 5), shows a decided decrease in the aspen-fir type as compared with that in the oak-brush type. In the spruce-fir type the evaporation is almost as great as in the oak-brush type, largely because of the fact that the climatological station in this type is situated on an open southern aspect which

is exposed to the full sweep of the prevailing southwest winds, the velocity of which Sampson (113) has shown to average notably greater than in the lower types. Because of the prevailing high temperatures, the longer growing season, and the higher rates of evaporation in the lower types it follows that the mean annual precipitation is less effective in these types. Even in the oak-brush zone the rainfall is insufficient to leach the lime from the soil. This complex of adverse factors, therefore, has a profound influence on the type of vegetation at the lower elevations.<sup>12</sup>

TABLE V.—*Soil moisture and evaporation data on six different sites at elevations of 7,500 to 7,700 feet, Big Cottonwood watershed, Wasatch National Forest, Utah, season of 1919.*

Site	Depth of soil sample	Total soil moisture percentages at different dates of sampling								Wilt- ing co- effi- cient <sup>a</sup>	Mean daily evap- ora- tion	E. <sup>b</sup> S. M.
		July 2	July 12	July 29	Aug- ust 9	Aug- ust 20	Aug- ust 30	Sept- ember 22	Sea- sonal average			
Northern aspect, brushy.	<i>Inches</i>									<i>Per cent</i>	<i>c. c.</i>	
	0-6	13.2	10.5	5.2	9.8	7.8	7.4	23.4	11.0	13.6		
	6-12	12.4	12.8	7.6	10.9	9.5	8.6	18.5	11.5	11.3	21.29	1.85
	Average..	12.8	11.6	6.4	10.4	8.6	8.0	21.0	11.2	12.4		
Western aspect, Ceanothus-oak.	0-6	4.4	5.0	2.7	19.8	5.8	3.0	11.3	7.4	} 10.9	33.46	3.80
	6-12	7.5	8.1	5.4	17.3	6.6	4.6	12.2	8.8			
	Average..	6.0	6.6	4.0	18.6	6.2	3.8	11.8	8.1			
Northeastern aspect open, grassy.	0-6	7.9	4.8	3.6	21.0	8.3	5.6	30.4	11.7	17.9		
	6-12	9.8	9.9	8.5	14.0	9.6	8.8	24.6	12.2	14.5	30.07	2.46
	Average..	8.8	7.4	6.0	17.5	9.0	7.2	27.5	12.0	16.2		
Northeastern aspect, aspen.	0-6	14.9	15.1	7.6	16.0	10.4	9.2	19.6	13.3	14.7		
	6-12	15.5	16.7	8.6	20.8	10.2	10.6	16.6	14.1	12.6	(c)	c 1.51
	Average..	15.2	15.9	8.1	18.4	10.3	9.9	18.1	13.7	13.6		
Canyon Bottom, scattered aspen.	0-6	10.0	13.0	7.8	24.4	9.2	8.4	19.8	13.2	16.2		
	6-12	8.8	12.7	9.2	19.3	11.2	8.7	16.6	12.4	13.5	24.45	1.97
	Average..	9.4	12.8	8.5	21.8	10.2	8.6	18.2	12.8	14.8		
Southern aspect, sage-Ceanothus-oak-aspen.	0-6	3.0	3.1	4.0	20.4	6.1	2.6	8.4	6.8	} 12.4	38.27	3.99
	6-12	7.7	6.2	7.0	20.6	8.5	5.8	11.4	9.6			
	Average..	5.4	4.6	5.5	20.5	7.3	4.2	9.9	8.2			

<sup>a</sup> Determined in the Biophysical Laboratory, Bureau of Plant Industry, United States Department of Agriculture by the Briggs and Shantz centrifugal method.

<sup>b</sup> Evaporation divided by the mean seasonal moisture content of the soil at a depth of 6-12 inches.

<sup>c</sup> Atometer was broken by a bear early in the season. In computing the ratio of evaporation to the soil moisture content, the evaporation was considered equal to that on the brushy northern aspect. This is shown to be ultra-conservative by the 1920 records which show the evaporation to be lower on the aspen-covered northeastern aspect.

<sup>12</sup> Studies conducted in the Wasatch Mountains by Sampson (113) and Baker and Korstian (unpublished) agree in the main with the more detailed studies of Pearson (99) in indicating that the upper limits of the distribution of a species or forest type are determined primarily by low temperature as related to photo-synthetic activity and that the lower limits are determined primarily by a deficient moisture supply. Deficient moisture rather than high temperature is regarded as the determinant at the lower levels, since the observations by Baker and Korstian in this region, as well as those of Pearson in the Southwest, indicate that practically all of the species, when adequately supplied with moisture through irrigation or when growing along stream courses, some 1,500 feet below their natural range, are capable of enduring high temperatures far in excess of those to which they are subjected at the lower limits of their natural range.

TABLE VI.—*Soil moisture and evaporation data on six different sites at elevations of 7,500 to 7,700 feet on Big Cottonwood watershed, Wasatch National Forest, Utah, season of 1920*

Site	Depth of soil sample	Total soil moisture at different dates of sampling									
		June 9	June 16	June 23	July 3	July 14	July 29	August 9	August 18	August 30	September 28
	Inches	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Northern aspect, brushy----	0-6	9.0	6.0	6.1	5.1	3.6	9.1	14.2	8.8	17.0	21.5
	6-12	10.9	8.1	8.6	15.0	6.6	9.8	10.2	9.5	12.1	23.2
	Average---	10.0	7.0	7.4	10.0	5.1	9.4	12.2	9.2	14.6	22.4
Western aspect, Ceanothus-oak.	0-6	10.0	11.5	5.7	4.2	4.4	7.9	9.2	9.3	18.0	20.5
	6-12	10.0	15.2	9.9	7.8	4.4	5.7	7.1	7.6	12.9	13.5
	Average---	10.0	13.4	7.8	6.0	4.4	6.8	8.2	8.4	15.4	17.0
Northeastern aspect, open, grassy.	0-6	20.2	16.6	8.8	9.3	4.8	10.1	16.6	11.0	30.9	35.4
	6-12	23.0	21.2	13.5	12.1	10.3	9.7	11.1	11.7	28.2	30.1
	Average---	21.6	18.9	11.2	10.7	7.6	9.9	13.8	11.4	29.6	32.8
Northeastern aspect, aspen.	0-6	28.9	23.9	20.8	19.5	10.2	18.6	21.7	9.4	28.1	36.5
	6-12	24.7	27.1	23.1	16.6	12.2	13.2	14.2	10.4	16.2	25.5
	Average---	26.8	25.5	22.0	18.0	11.2	15.9	18.0	9.9	22.2	31.0
Canyon bottom, scattered aspen.	0-6	13.8	11.3	10.0	7.5	5.5	12.3	18.1	8.5	24.3	23.5
	6-12	27.9	13.3	15.4	13.5	7.3	9.8	12.5	9.4	19.5	21.6
	Average---	20.8	12.3	12.7	10.5	6.4	11.0	15.3	9.0	21.9	22.6
Southern aspect, sage-Ceanothus-oak-aspen.	0-6	7.4	5.4	6.6	6.9	3.4	11.0	14.5	6.0	15.8	17.2
	6-12	16.4	11.7	11.5	10.2	6.5	9.1	11.1	7.6	13.8	19.4
	Average---	11.9	8.6	9.0	8.6	5.0	10.0	12.8	6.8	14.8	18.3

Site	Depth of soil sample	Seasonal average of soil moisture	Wilting coefficient <sup>a</sup>	Mean daily evaporation	$\frac{E^b}{S. M.}$	$\frac{E^b}{S. M.}$ mean of 1919 and 1920
Northern aspect, brushy-----	Inches	Per cent	Per cent	Cc.		
	0-6	10.0	13.6			
	6-12	11.4	11.3	10.86	0.95	1.40
Western aspect, Ceanothus-oak-----	Average---	10.7	12.4			
	0-6	10.1	10.9	18.65	1.98	2.89
	6-12	9.4				
Northeastern aspect, open, grassy-----	Average---	9.8				
	0-6	16.4	17.9			
	6-12	17.1	14.5	15.12	0.88	1.67
Northeastern aspect, aspen-----	Average---	16.8	16.2			
	0-6	21.8	14.7			
	6-12	13.3	12.6	9.96	0.54	1.02
Canyon bottom, scattered aspen-----	Average---	20.0	13.6			
	0-6	13.5	16.2			
	6-12	15.0	13.5	19.13	1.28	1.62
Southern aspect, sage-Ceanothus-oak-aspen.	Average---	14.2	14.8			
	0-6	9.4	12.4	20.90	1.79	2.89
	6-12	11.7				
	Average---	10.6				

• Determined in the Biophysical Laboratory, Bureau of Plant Industry, United States Department of Agriculture by the Briggs and Shantz centrifugal method.  
<sup>b</sup> Evaporation divided by the mean seasonal moisture content of the soil at a depth of 6-12 inches.

SAP-DENSITY DATA

The object of this study was to attempt a correlation of the major vegetational types with the cell-sap density of representative plants. The procedure therefore involved a systematic sampling of each of the well-marked types. In the Ephraim Canyon investigation six such types were recognized: (1) greasewood-shadscale, (2) sagebrush, (3) pinion-juniper, (4) oak brush, (5) aspen-fir, (6) spruce-fir. In the main Wasatch Mountains eight major vegetational types or associations were considered: (1) stream-bank, (2) sagebrush, (3) juniper, (4) western yellow pine, (5) chaparral, (6) lodgepole pine, (7) aspen-fir, (8) spruce-fir. With the exception of the stream-bank association, these types form

a regularly graduated succession from the xerophytic sagebrush to the climax mesophytic spruce-fir type.<sup>13</sup> These types were further divided into sites, differentiated chiefly on the basis of aspect and consequently varying as to moisture supply, rate of evaporation, temperature, and exposure to sunlight. The possible effect which altitude might have upon sap density was also taken into account in selecting some of the sites on which the various species were collected.

In the Central Wasatch Mountains some 500 sap density determinations were made on representative trees, shrubs, and herbs of the above-mentioned types. The samples were collected in Big Cottonwood and Taylor Canyons, lying near Salt Lake City and Ogden, Utah, respectively. In the Ephraim Canyon series of July, 1921, approximately 275 determinations were made of the sap densities of plants on the west slope of the Wasatch Plateau and on the desert in the adjacent San Pete Valley.

TABLE VII.—*Freezing point depressions and osmotic pressures of the sap of trees classified by species, locality, type, situation, altitude, and date of collection*

[Numbers in italics represent averages of two or more determinations]

Species and locality	Type	Situation	Altitude	Date of collection	Depression of freezing point	Osmotic pressure
<i>Juniperus utahensis</i> (Engelm.) Lemm.					<i>Degrees C.</i>	<i>Atmospheres</i>
Big Cottonwood Canyon.	Juniper	Southern aspect	<i>Feet</i> 5,000	1920-1921 July 21	<i>2.19</i>	<i>26.3</i>
Do.	do.	do.	5,000	Sept. 17	<i>1.32</i>	<i>15.9</i>
Do.	do.	do.	5,000	Dec. 10	<i>2.31</i>	<i>27.8</i>
Taylor Canyon.	do.	do.	5,200	Jan. 4	<i>2.12</i>	<i>25.5</i>
Do.	do.	do.	5,200	Feb. 28	<i>2.14</i>	<i>25.7</i>
Do.	do.	do.	7,000	do.	<i>2.35</i>	<i>28.2</i>
<i>Pinus ponderosa scopulorum</i> Engelm.						
Big Cottonwood Canyon.	Western yellow pine	Southern aspect	6,400	July 21	<i>1.93</i>	<i>23.2</i>
Do.	do.	do.	6,400	Sept. 22	<i>1.44</i>	<i>17.3</i>
Do.	do.	do.	6,400	Dec. 10	<i>1.38</i>	<i>16.6</i>
Near Park City highway.	Isolated trees	Eastern aspect	6,800	Sept. 17	<i>1.47</i>	<i>17.7</i>
Beaver Creek.	Western yellow pine	Southern aspect	7,000	Sept. 16	<i>1.86</i>	<i>22.4</i>
Do.	do.	do.	7,300	do.	<i>1.82</i>	<i>21.9</i>
Do.	do.	do.	7,600	do.	<i>1.77</i>	<i>21.3</i>
Big Cottonwood Canyon.	Aspen-fir	Canyon bottom <sup>a</sup>	7,500	July 7	<i>1.57</i>	<i>18.9</i>
Do.	do.	do.	7,500	July 27	<i>1.35</i>	<i>16.2</i>
Do.	do.	Southern aspect, under light shade. <sup>a</sup>	7,500	July 19	<i>1.36</i>	<i>16.4</i>
Do.	do.	do.	7,500	July 27	<i>1.35</i>	<i>16.2</i>
Do.	do.	Canyon bottom <sup>a</sup>	7,500	Sept. 20	<i>1.36</i>	<i>16.4</i>
Do.	do.	Canyon bottom under snow.	7,500	Dec. 8	<i>1.17</i>	<i>14.1</i>
<i>Juniperus scopulorum</i> Sarg.						
Big Cottonwood Canyon.	Western yellow pine	Southern aspect	6,400	July 21	<i>2.41</i>	<i>28.9</i>
Do.	Chaparral	do.	7,600	July 26	<i>1.60</i>	<i>19.2</i>
Do.	Western yellow pine	do.	6,400	Sept. 22	<i>0.99</i>	<i>11.9</i>
Do.	Chaparral	do.	7,600	Sept. 7	<i>0.94</i>	<i>11.3</i>
Do.	do.	do.	7,600	Sept. 10	<i>0.92</i>	<i>11.1</i>
Do.	Western yellow pine	do.	6,400	Dec. 10	<i>2.41</i>	<i>28.9</i>
Do.	Chaparral	do.	7,600	Dec. 6	<i>2.17</i>	<i>26.1</i>

<sup>a</sup> Planted, species exotic to this site.

<sup>c</sup> Sample collected after a heavy rain.

<sup>13</sup> These types are not essentially different from the successional formations of Clements (20, 21). His two recent monographs constitute a thorough treatment of the purely successional aspects of the type problem. Here the author has brought together his extensive observations on the relations of vegetation to environmental conditions; and has shown that the changes which occur in the character of the vegetation of a given plant association are the result of gradual changes in climate and soil conditions and reactions of the plants upon the environment.

TABLE VII.—Freezing point depressions and osmotic pressures of the sap of trees classified by species, locality, type, situation, altitude, and date of collection—Con.

[Numbers in italics represent averages of two or more determinations]

Species and locality	Type	Situation	Altitude	Date of collection	Depression of freezing point	Osmotic pressure
<i>Juniperus scopulorum</i> Sarg.			<i>Feet</i>	<i>1920-1921</i>	<i>Degrees C.</i>	<i>Atmospheres</i>
Beaver Creek	Western yellow pine	Southern aspect	7,000	Sept. 16	1.40	16.8
Do	do	do	7,300	do	1.55	18.6
Do	do	do	7,600	do	1.39	16.7
Ogden, Utah	do	City park <sup>a</sup>	4,350	Jan. 25	2.23	26.8
Taylor Canyon	Aspen-fir	Northern aspect	5,200	Jan. 4	2.20	26.4
Do	Juniper	Southern aspect	6,600	do	2.06	24.8
Do	do	do	7,600	do	2.01	24.2
Do	Aspen-fir	Northern aspect	5,200	Feb. 28	1.75	21.0
Do	Juniper	Southern aspect	8,500	do	2.84	34.1
<i>Pseudotsuga taxifolia</i> (Lam.) Britton						
Big Cottonwood Canyon.	Western yellow pine	Southern aspect	6,400	July 21	1.74	20.9
Do	Aspen-fir	Canyon bottom	7,400	July 23	1.16	14.0
Do	do	do	7,400	July 25	1.36	16.4
Do	do	Southern aspect	7,500	July 19	1.62	19.5
Do	do	do	7,500	July 26	1.54	18.5
Do	do	Northern aspect	7,600	July 13	1.62	19.5
Do	do	do	7,700	July 19	1.70	20.4
Do	Spruce-fir	do	8,500	July 24	1.58	19.0
Do	Western yellow pine	Southern aspect	6,400	Sept. 22	1.41	17.0
Do	Aspen-fir	do	7,500	Sept. 7	1.32	15.9
Do	do	do	7,500	Sept. 10	0.90	10.8
Do	do	do	7,500	Sept. 12	1.15	13.8
Do	do	Northern aspect	7,700	Sept. 7	1.41	17.0
Do	do	do	8,250	Sept. 20	1.04	12.5
Do	do	do	8,500	Sept. 12	1.18	14.2
Do	Aspen-fir	Western aspect	8,500	Sept. 20	0.98	11.8
Do	do	do	9,600	do	1.07	12.9
Do	do	Southern aspect	10,000	Sept. 9	0.89	10.7
Do	Western yellow pine	do	6,400	Dec. 10	1.22	15.9
Do	Aspen-fir	do	7,500	Dec. 6	1.24	14.9
Do	do	Northern aspect	7,600	Dec. 7	1.32	15.9
Do	do	Western aspect	9,500	do	1.05	12.6
Beaver Creek	do	Northern aspect	7,000	Sept. 17	0.79	9.5
Do	do	Eastern aspect	7,300	Sept. 16	1.75	21.0
Do	do	do	7,400	do	1.75	21.0
Do	do	do	7,600	do	1.62	19.5
Taylor Canyon	do	Northern aspect	5,200	Jan. 4	0.57	6.9
Do	do	do	7,800	do	0.57	6.9
Do	do	do	5,200	Feb. 28	0.74	8.9
Do	do	do	7,000	do	0.66	8.0
Do	do	do	8,500	do	0.66	8.0
Big Cottonwood Canyon.	do	Canyon bottom <sup>b</sup>	7,400	July 27	1.59	19.1
Do	do	do	7,400	Sept. 22	1.50	18.0
Ogden, Utah	do	City park <sup>a</sup>	4,350	Jan. 25	0.92	11.1
<i>Abies concolor</i> (Gord.) Parry						
Big Cottonwood Canyon.	Aspen-fir	Northern aspect	5,500	July 21	1.66	20.0
Do	Western yellow pine	Southern aspect	6,400	do	1.96	23.6
Do	Aspen-fir	do	7,500	July 19	1.30	15.6
Do	do	do	7,500	July 26	1.35	16.2
Do	do	Northern aspect	7,600	July 13	1.71	20.6
Do	do	do	7,700	July 19	1.52	18.3
Do	Western yellow pine	Southern aspect	6,400	Sept. 22	1.15	13.8
Do	Aspen-fir	do	7,500	Sept. 7	1.32	15.9
Do	do	do	7,500	Sept. 10	0.93	11.2
Do	do	do	7,500	Sept. 12	1.07	12.9
Do	do	Northern aspect	7,700	Sept. 7	1.20	14.4
Do	do	do	8,250	Sept. 20	1.04	12.5
Do	do	Ridge top	8,650	do	1.19	14.3
Do	Western yellow pine	Southern aspect	6,400	Dec. 10	0.83	10.0
Do	Aspen-fir	do	7,500	Dec. 6	1.35	16.2
Do	do	Northern aspect	7,600	Dec. 7	1.28	15.4
Do	do	Ridge top	8,000	do	1.47	17.7

<sup>a</sup> Planted, species exotic to this site.<sup>b</sup> Planted although native to site.<sup>c</sup> Sample collected after a heavy rain.

TABLE VII.—Freezing point depressions and osmotic pressures of the sap of trees classified by species, locality, type, situation, altitude, and date of collection—Con.

[Numbers in italics represent averages of two or more determinations]

Species and locality	Type	Situation	Altitude	Date of collection	Depression of freezing point	Osmotic pressure
<i>Abies concolor</i> (Gord.) Parry			<i>Feet</i>	<i>1920-1921</i>	<i>Degrees C.</i>	<i>Atmospheres</i>
Beaver Creek	Aspen-fir	Eastern aspect	7,400	Sept. 16	1.28	15.4
Taylor Canyon	do	Northern aspect	5,200	Jan. 4	0.74	8.9
Do	do	do	7,800	do	0.66	8.0
Ogden, Utah		City park <sup>a</sup>	4,350	Jan. 25	1.04	12.5
Taylor Canyon	Aspen-fir	Northern aspect	5,200	Feb. 28	0.98	11.8
Do	do	do	7,000	do	1.06	12.8
Do	do	do	8,500	do	0.64	7.7
<i>Picea parryana</i> (André) Parry						
Big Cottonwood Canyon.	Aspen-fir	Canyon bottom	7,000	July 23	1.26	15.2
Do	do	do	7,200	July 19	1.37	16.5
Do	do	do	7,400	July 13	1.42	17.1
Do	do	do	7,400	July 23	1.11	13.4
Do	do	do	7,400	July 25	1.11	13.4
Do	do	Lower southern aspect	7,450	July 26	1.23	14.8
Do	do	Canyon bottom	7,700	July 24	1.16	14.0
Do	do	do	7,000	Sept. 20	0.91	11.0
Do	do	do	7,200	Sept. 22	1.00	12.0
Do	do	Lower southern aspect	7,450	Sept. 7	1.14	13.7
Do	do	Canyon bottom	7,000	Dec. 10	1.02	12.3
Do	do	do	7,200	Dec. 6	1.34	16.1
Do	do	Lower southern aspect	7,450	Dec. 8	1.40	16.8
Beaver Creek	do	Canyon bottom	7,600	Sept. 16	1.19	14.3
Big Cottonwood Canyon.	do	do <sup>b</sup>	7,400	July 11	1.27	15.3
Ogden, Utah		City park <sup>a</sup>	4,350	Jan. 25	1.16	14.0
<i>Picea engelmanni</i> (Parry) Engelm.						
Big Cottonwood Canyon.	Aspen-fir	Canyon bottom	7,200	July 19	1.09	13.1
Do	do	do	7,400	July 13	1.45	17.4
Do	do	Northern aspect	7,500	July 27	1.15	13.8
Do	Spruce-fir	do	8,500	July 24	1.58	19.0
Do	do	do	8,800	July 25	1.51	18.2
Do	do	Ridge top	10,000	July 15	1.53	19.0
Do	Aspen-fir	Canyon bottom	7,200	Sept. 22	1.13	13.6
Do	do	Northern aspect	8,250	Sept. 20	0.85	10.7
Do	Spruce-fir	do	8,500	Sept. 12	0.97	11.2
Do	do	do	9,600	Sept. 20	1.18	14.2
Do	do	do	10,000	Sept. 9	1.12	13.5
Do	Aspen-fir	Canyon bottom	7,200	Dec. 6	1.29	15.5
Do	do	Northern aspect	7,500	Dec. 7	1.40	16.8
Do	Spruce-fir	do	9,500	do	0.95	11.4
Beaver Creek	Aspen-fir	Canyon bottom	7,600	Sept. 16	1.43	17.2
Big Cottonwood Canyon.	do	do <sup>b</sup>	7,400	July 11	1.32	15.9
Do	do	do <sup>b</sup>	7,400	July 25	1.28	15.4
<i>Abies lasiocarpa</i> (Hook.) Nutt						
Big Cottonwood Canyon.	Aspen-fir	Canyon bottom	7,200	July 19	1.10	13.2
Do	do	do	7,400	July 13	1.45	17.4
Do	do	Northern aspect	7,500	July 12	1.09	13.1
Do	do	Lower southern aspect	7,450	July 26	0.88	10.6
Do	do	do	7,450	July 27	0.48	5.8
Do	do	Northern aspect	7,700	July 19	1.51	18.2
Do	Spruce-fir	do	8,500	July 24	1.53	18.4
Do	do	do	8,800	July 25	1.26	15.2
Do	do	Ridge top	10,000	July 15	1.54	18.5
Do	Aspen-fir	Canyon bottom	7,200	Sept. 22	0.75	9.0
Do	do	Lower southern aspect	7,450	Sept. 7	0.83	10.0
Do	do	Northern aspect	7,700	do	1.05	12.6
Do	do	do	8,250	Sept. 20	0.83	10.0
Do	do	Western aspect	9,600	do	0.99	11.9

<sup>a</sup> Planted, species exotic to this site.<sup>b</sup> Planted although native to site.



TABLE VII.—Freezing point depressions and osmotic pressures of the sap of trees classified by species, locality, type, situation, altitude, and date of collection—Con.

[Numbers in italics represent averages of two or more determinations]

Species and locality	Type	Situation	Altitude	Date of collection	Depression of freezing point	Osmotic pressure
<i>Abies lasiocarpa</i> (Hook.) Nutt.—Con.			<i>Feet</i>	<i>1920-1921</i>	<i>Degrees C.</i>	<i>Atmospheres</i>
Big Cottonwood Canyon.	Spruce-fir	Northern aspect	8,500	Sept. 12	0.74	8.9
Do.	do	do	9,600	Sept. 20	0.98	11.8
Do.	do	do	10,000	Sept. 9	0.72	8.7
Do.	Aspen-fir	Canyon bottom	7,200	Dec. 6	0.86	10.4
Do.	do	Lower southern aspect	7,450	Dec. 8	1.35	16.2
Do.	do	Northern aspect	7,600	Dec. 7	1.30	15.6
Do.	do	Western aspect	9,500	do	0.85	10.2
Do.	Spruce-fir	Northern aspect	9,500	do	1.12	13.5
Beaver Creek	Aspen-fir	Eastern aspect	7,400	Sept. 16	0.87	10.5
Do.	do	Canyon bottom	7,500	do	1.03	12.4
Taylor Canyon	Spruce-fir	Northern aspect	7,800	Jan. 4	0.42	5.1
Do.	do	do	8,500	Feb. 28	0.74	8.9
<i>Pinus flexilis</i> James						
Big Cottonwood Canyon.	Western yellow pine	Southern aspect	6,400	July 21	1.74	20.9
Do.	Aspen-fir	Northern aspect	7,500	July 13	1.27	15.3
Do.	do	Southern aspect	10,000	July 15	1.92	23.1
Do.	Western yellow pine	do	6,400	Sept. 22	1.16	14.0
Do.	Aspen-fir	Northern aspect	7,700	Sept. 7	1.24	14.9
Do.	do	do	8,250	Sept. 20	1.23	14.8
Do.	do	Western aspect	9,600	do	1.04	12.5
Do.	Spruce-fir	Northern aspect	10,000	Sept. 9	0.92	11.1
Do.	Western yellow pine	Southern aspect	6,400	Dec. 10	1.14	13.7
Do.	Aspen-fir	Northern aspect	7,700	Dec. 7	0.99	11.9
Do.	do	Western aspect	9,500	do	1.29	15.5
Beaver Creek	do	Southern aspect	7,000	Sept. 16	1.64	19.7
Do.	do	Northern aspect	7,000	Sept. 17	0.89	10.7
Taylor Canyon	do	Southern aspect	7,600	Jan. 4	0.79	9.5
Do.	do	do	8,500	Feb. 28	0.54	6.5
Do.	do	Northern aspect	8,500	do	0.67	8.1
<i>Pinus contorta</i> Loud.						
Beaver Creek	Lodgepole pine	Bench in canyon bottom.	7,000	Sept. 16	1.46	17.6
Do.	do	do	7,300	do	1.52	18.3
Do.	do	Flat divide	7,500	do	1.39	16.7
Do.	do	Bench in canyon bottom.	7,300	do	1.68	20.2
Big Cottonwood Canyon.	Aspen-fir	Canyon bottom <sup>a</sup>	7,400	July 7	1.42	17.1
Do.	do	do	7,400	July 12	1.44	17.3
Do.	do	do	7,400	Dec. 8	1.48	17.8
<i>Pinus ponderosa</i> Laws.						
Big Cottonwood Canyon.	Aspen-fir	Southern aspect <sup>a</sup>	7,200	July 23	1.15	13.8
Do.	do	do	7,200	Dec. 10	1.11	13.4
Do.	Chaparral	do	7,300	July 25	1.49	17.9
Do.	Aspen-fir	do	7,450	July 27	1.05	12.6
Do.	do	do	7,500	July 19	1.43	17.2
Do.	do	Canyon bottom <sup>a</sup>	7,400	July 7	1.55	18.6
Do.	do	do	7,400	July 27	1.35	16.2
Do.	do	do	7,400	Sept. 20	1.37	16.5
Do.	do	Canyon bottom under 3 feet snow <sup>a</sup>	7,400	Dec. 8	1.34	16.1
Do.	do	Canyon bottom <sup>a</sup>	7,400	do	1.83	22.0
Do.	do	do	7,400	July 13	1.20	14.4
Do.	do	do	7,400	July 14	1.16	14.0
Do.	do	Northern aspect <sup>a</sup>	7,450	July 19	1.42	17.1
Do.	do	Canyon bottom <sup>a</sup>	7,400	Sept. 22	0.97	11.7
Do.	do	do	7,400	Dec. 8	1.25	15.0
Ogden, Utah	do	City park <sup>a</sup>	4,350	Jan. 25	0.75	9.0
<i>Pinus austriaca</i> Höss.						
Ogden, Utah	do	City park <sup>a</sup>	4,350	do	1.19	14.3

<sup>a</sup> Planted, species exotic to this site.<sup>b</sup> Planted although native to site.<sup>c</sup> Sample collected after a heavy rain.

TABLE VII.—Freezing point depressions and osmotic pressures of the sap of trees classified by species, locality, type, situation, altitude, and date of collection—Con.

[Numbers in italics represent averages of two or more determinations]

Species and locality	Type	Situation	Altitude	Date of collection	Depression of freezing point	Osmotic pressure
<i>Pinus banksiana</i> Lamb.					<i>Degrees C.</i>	<i>Atmospheres</i>
Big Cottonwood Canyon.	Aspen-fir.....	Northern aspect <sup>a</sup> .....	<i>Feet</i> 7,450	1920-1921 July 27	1.07	12.9
<i>Pinus laricio corsicana</i> Hort.						
Big Cottonwood Canyon.	Aspen-fir.....	Northern aspect <sup>a</sup> .....	7,450	July 27	1.28	15.4
<i>Pinus jeffreyi</i> Murr.						
Big Cottonwood Canyon.	Aspen-fir.....	Northern aspect <sup>a</sup> .....	7,450	July 19	1.38	16.6
<i>Pinus montana</i> Mill.						
Ogden, Utah.....		City park <sup>a</sup> .....	4,350	Jan. 25	0.90	10.8
<i>Pinus monticola</i> Dougl.						
Big Cottonwood Canyon.	Aspen-fir.....	Canyon bottom <sup>a</sup> .....	7,400	July 7	1.41	17.0
Do.....	do.....	Northern aspect <sup>a</sup> .....	7,450	July 23	1.15	13.8
<i>Pinus strobus</i> L.						
Ogden, Utah.....		City park <sup>a</sup> .....	4,350	Jan. 25	1.23	14.8
<i>Picea excelsa</i> Link.						
Big Cottonwood Canyon.	Aspen-fir.....	Canyon bottom <sup>a</sup> .....	7,400	July 11	1.35	16.2
Do.....	do.....	do.....	7,400	July 23	1.18	14.2
Ogden, Utah.....		City park <sup>a</sup> .....	4,350	Jan. 25	0.93	11.2
<i>Larix occidentalis</i> Nutt.						
Big Cottonwood Canyon.	Aspen-fir.....	Northern aspect <sup>a</sup> .....	7,450	July 19	1.60	19.2
<i>Thuja occidentalis</i> L.						
Ogden, Utah.....		City park <sup>a</sup> .....	4,350	Jan. 25	2.72	32.6
<i>Thuja orientalis</i> L.						
Ogden, Utah.....		City park <sup>a</sup> .....	4,350	do.....	1.73	20.8
<i>Acer grandidentatum</i> Nutt.						
Big Cottonwood Canyon.	Stream-bank.....	Canyon bottom.....	4,500	July 21	1.64	19.7
Do.....	Western yellow pine.....	Southern aspect.....	6,400	do.....	1.83	22.0
Do.....	do.....	do.....	6,400	Sept. 22	1.44	17.3
Do.....	Aspen-fir.....	Lower southern aspect.....	7,450	July 26	1.43	17.2
Do.....	do.....	do.....	7,450	Sept. 7	1.44	17.3
Do.....	do.....	Northwestern aspect.....	7,700	Sept. 9	1.52	18.3
<i>Acer glabrum</i> Torr.						
Big Cottonwood Canyon.	Aspen-fir.....	Northern aspect.....	7,500	July 13	1.17	14.1
<i>Acer negundo</i> L.						
Big Cottonwood Canyon.	Stream-bank.....	Canyon bottom.....	4,300	July 18	0.09	13.1
Do.....	do.....	do.....	4,400	July 21	1.09	13.1
Do.....	do.....	do.....	6,800	do.....	1.06	12.8

<sup>a</sup> Planted, species exotic to this site.

TABLE VII.—Freezing point depressions and osmotic pressures of the sap of trees classified by species, locality, type, situation, altitude, and date of collection—Con.

[Numbers in italics represent averages of two or more determinations]

Species and locality	Type	Situation	Altitude	Date of collection	Depression of freezing point	Osmotic pressure
<i>Populus angustifolia</i> James			<i>Feet</i>	<i>1920-1921</i>	<i>Degrees C.</i>	<i>Atmospheres</i>
Big Cottonwood Canyon.	Stream-bank.....	Canyon bottom.....	4,300	July 8	1.46	17.6
Do.....	do.....	do.....	4,400	July 21	1.37	16.5
Do.....	Western yellow pine.	Southern aspect.....	6,400	do.....	1.66	20.0
Do.....	Aspen-fir.....	Canyon bottom.....	7,400	July 14	1.54	18.5
Do.....	do.....	do.....	7,400	July 22	1.67	20.1
<i>Populus tremuloides</i> Michx.						
Big Cottonwood Canyon.	Aspen-fir.....	Canyon bottom.....	7,450	July 11	1.82	21.9
Do.....	do.....	Southern aspect.....	7,500	July 19	2.26	27.2
Do.....	do.....	do.....	7,450	Sept. 6	2.37	28.5
Do.....	do.....	Northern aspect.....	7,500	July 13	1.74	20.9
Do.....	do.....	do.....	7,700	Sept. 7	2.02	24.3
Do.....	do.....	do.....	7,700	Sept. 9	1.83	22.0
Do.....	Spruce-fir.....	Ridge-top.....	10,000	July 15	1.82	23.1
Do.....	do.....	Northern aspect.....	10,000	Sept. 9	2.09	25.1
Beaver Creek.....	Lodgepole pine.....	Bench in canyon bottom.	7,400	Sept. 16	2.27	27.3
<i>Alnus tenuifolia</i> Nutt.						
Big Cottonwood Canyon.	Aspen-fir.....	Northern aspect.....	7,500	July 13	1.12	13.5
<i>Betula fontinalis</i> Sarg.						
Big Cottonwood Canyon.	Stream-bank.....	Canyon bottom.....	4,300	July 8	1.36	16.4
Do.....	Western yellow pine.	Southern aspect.....	6,400	July 21	1.50	18.0
<i>Crataegus rivularis</i> Nutt.						
Big Cottonwood Canyon.	Stream-bank.....	Canyon bottom.....	4,400	do.....	1.85	22.2

TABLE VIII.—Freezing point depressions and osmotic pressures of the sap of shrubs classified by species, locality, type, situation, altitude, and date of collection

[Figures in italics represent averages of two or more determinations]

Species and locality	Type	Situation	Altitude	Date of collection	Depression of freezing point	Osmotic pressure
<i>Artemisia tridentata</i> Nutt.			<i>Feet</i>	<i>1920-1921</i>	<i>Degrees C.</i>	<i>Atmospheres</i>
Big Cottonwood Canyon.	Stream bank.....	Canyon bottom.....	4,300	July 8	1.36	16.4
Do.....	Sagebrush.....	Southern aspect.....	5,000	July 21	2.51	30.1
Do.....	Chaparral.....	do.....	7,600	July 19	1.66	20.0
Do.....	Aspen-fir.....	Northern aspect.....	7,450	July 17	1.27	15.3
Do.....	Sagebrush.....	Southern aspect.....	5,000	Sept. 17	2.08	25.0
Do.....	Chaparral.....	do.....	7,600	Sept. 6	1.82	21.9
Do.....	Aspen-fir.....	Northern aspect.....	8,500	Sept. 20	1.41	17.0
Do.....	Sagebrush.....	Southern aspect.....	5,000	Dec. 10	2.06	24.8
Do.....	Chaparral.....	do.....	7,500	Dec. 6	2.69	32.3
Do.....	Aspen-fir.....	Ridge top.....	9,000	Dec. 7	1.97	23.7
Beaver Creek.....	Sagebrush.....	Southern aspect.....	7,400	Sept. 16	2.73	32.8
Taylor Canyon.....	do.....	Very gentle western aspect.	4,500	Jan. 4	2.85	34.2
Do.....	do.....	Southern aspect.....	5,200	do.....	3.34	40.0
Do.....	do.....	Very gentle western aspect.	4,500	Feb. 28	2.36	28.3
Do.....	do.....	Southern aspect.....	5,200	do.....	2.40	28.8
Do.....	do.....	do.....	7,000	do.....	2.31	27.8

TABLE VIII.—Freezing point depressions and osmotic pressures of the sap of shrubs classified by species, locality, type, situation, altitude, and date of collection—Con.

[Figures in italics represent averages of two or more determinations]

Species and locality	Type	Situation	Altitude	Date of collection	Depression of freezing point	Osmotic pressure
<i>Purshia tridentata</i> (Pursh) D. C.			<i>Feet</i>	<i>1920-1921</i>	<i>Degrees C.</i>	<i>Atmospheres</i>
Big Cottonwood Canyon.	Juniper.....	Southern aspect.....	5,000	July 21	1.89	22.7
Beaver Creek.....	Sagebrush.....	do.....	7,400	Sept. 16	2.15	25.8
<i>Gutierrezia filifolia</i> Greene						
Big Cottonwood Canyon.	Sagebrush.....	do.....	5,000	Sept. 17	2.55	30.6
<i>Rhus trilobata</i> Nutt.						
Big Cottonwood Canyon	Stream bank.....	Canyon bottom.....	4,300	July 8	1.57	18.9
Do.....	Juniper.....	Southern aspect.....	5,000	July 21	1.82	21.9
<i>Rhus cismontana</i> Greene						
Big Cottonwood Canyon.	Stream bank.....	Canyon bottom.....	4,300	July 8	1.37	16.5
Do.....	do.....	Southern aspect.....	4,500	July 21	1.56	18.8
<i>Quercus utahensis</i> (DC.) Rydb.						
Big Cottonwood Canyon.	Stream bank.....	Canyon bottom.....	4,300	July 8	1.66	20.0
Do.....	do.....	Southern aspect.....	4,500	July 21	1.84	22.1
Do.....	Western yellow pine.....	Steep southern aspect.....	6,400	do.....	2.07	24.9
Do.....	Chaparral.....	Southern aspect.....	7,550	July 16	1.80	21.6
Do.....	Juniper.....	do.....	5,200	Sept. 17	1.90	22.8
Do.....	Western yellow pine.....	do.....	6,400	Sept. 22	1.68	20.2
Do.....	Chaparral.....	do.....	7,550	Sept. 6	1.95	23.4
Do.....	Aspen-fir.....	Northwestern aspect.....	7,700	Sept. 9	1.90	22.8
Beaver Creek.....	Chaparral.....	Southern aspect.....	7,400	Sept. 16	2.00	24.0
<i>Prunus melanocarpa</i> (A. Nels.) Rydb.						
Big Cottonwood Canyon.	Stream bank.....	Canyon bottom.....	4,300	July 8	1.81	21.8
Do.....	Chaparral.....	Southern aspect.....	4,500	July 21	2.00	24.0
Do.....	do.....	do.....	7,550	July 26	1.74	20.9
Do.....	Aspen-fir.....	Northern aspect.....	7,450	July 17	1.52	18.3
Do.....	Spruce-fir.....	Ridge top.....	10,000	July 15	1.95	23.4
Do.....	Chaparral.....	Southern aspect.....	7,550	Sept. 7	1.55	18.6
Do.....	do.....	do.....	7,550	Sept. 12	1.75	21.0
Do.....	Aspen-fir.....	Northwestern aspect.....	7,700	Sept. 9	1.87	22.5
Do.....	do.....	Northern aspect.....	7,450	Sept. 13	1.20	14.4
Do.....	Spruce-fir.....	Eastern aspect.....	9,000	Sept. 9	2.09	25.1
<i>Cercocarpus ledifolius</i> Nutt.						
Big Cottonwood Canyon.	Chaparral.....	Southern aspect.....	6,400	July 21	2.71	32.5
Do.....	do.....	do.....	7,550	July 16	1.50	18.0
Do.....	do.....	do.....	7,600	July 26	1.56	18.8
Do.....	do.....	do.....	6,400	Sept. 22	1.19	14.3
Do.....	do.....	do.....	7,550	Sept. 6	1.58	19.0
Do.....	do.....	do.....	6,400	Dec. 10	2.67	32.0
Do.....	do.....	do.....	7,550	Dec. 6	2.60	31.2
Do.....	do.....	do.....	5,200	Jan. 4	2.79	33.5
Taylor Canyon.....	do.....	do.....	7,600	do.....	2.99	35.9
Do.....	do.....	do.....	7,000	Feb. 28	2.66	31.9
Do.....	do.....	do.....	8,500	do.....	2.60	31.2
<i>Cercocarpus Montanus</i> Raf.						
Beaver Creek.....	Chaparral.....	Ridge top.....	7,100	Sept. 17	2.04	24.5

TABLE VIII.—Freezing point depressions and osmotic pressures of the sap of shrubs classified by species, locality, type, situation, altitude, and date of collection—Con.

[Figures in italics represent averages of two or more determinations]

Species and locality	Type	Situation	Altitude	Date of collection	Depression of freezing point	Osmotic pressure
<i>Sericotheca dumosa</i> (Nutt.) Rydb.					Degrees C.	Atmospheres
Big Cottonwood Canyon.	Chaparral	Southern aspect	6,400	1920-1921 July 21	3.13	37.5
Do.	do.	do.	6,400	Sept. 22	3.23	38.7
<i>Odostemon repens</i> (Lindl.) Cocker.						
Big Cottonwood Canyon.	Sagebrush	Southern aspect	4,500	July 21	1.98	23.8
Do.	Chaparral	do.	6,400	do.	2.51	30.1
Do.	Aspen-fir	Northern aspect	7,450	July 23	1.27	15.3
Do.	Spruce-fir	Ridge-top	10,000	July 15	1.46	17.6
Do.	Chaparral	Southern aspect	7,550	July 19	1.76	21.2
Do.	do.	do.	7,550	Sept. 7	1.60	19.2
Do.	do.	do.	7,550	Sept. 12	1.65	19.8
Do.	Aspen-fir	Northern aspect	7,450	Sept. 20	1.57	18.9
Do.	Chaparral	Southern aspect	6,400	Dec. 10	2.59	31.1
<i>Ceanothus velutinus</i> Dougl.						
Big Cottonwood Canyon.	Aspen-fir	Northern aspect	7,450	July 11	1.06	12.8
Do.	Chaparral	Southern aspect	7,550	July 16	1.35	16.2
Do.	Aspen-fir	Northern aspect	7,450	Sept. 20	1.46	17.6
Do.	Chaparral	Southern aspect	7,550	Sept. 6	1.84	22.1
Do.	Aspen-fir	Northwestern aspect	7,700	Sept. 9	0.96	11.6
Do.	Chaparral	Southern aspect	6,400	Dec. 10	3.03	36.4
Do.	do.	do.	7,550	Dec. 6	2.58	31.0
Beaver Creek	do.	do.	7,400	Sept. 16	2.10	25.2
Taylor Canyon	Aspen-fir	Northern aspect	5,200	Jan. 4	2.99	35.9
Do.	Chaparral	Southern aspect	5,200	Feb. 28	2.73	32.8
Do.	Aspen-fir	Northern aspect	5,200	do.	2.47	29.7
<i>Pachystima myrsinites</i> (Pursh) Raf.						
Big Cottonwood Canyon.	Aspen-fir	Northern aspect	7,500	July 13	1.33	16.0
Do.	Chaparral	Southern aspect	7,550	July 19	1.41	17.0
Do.	Aspen-fir	Northern aspect	7,450	Sept. 20	1.24	14.9
Do.	do.	Northwestern aspect	7,700	Sept. 9	1.16	14.0
Do.	Chaparral	Southern aspect	7,550	Sept. 7	1.38	16.6
Do.	Aspen-fir	Southern aspect (under shade).	7,450	Sept. 10	0.66	8.0
Do.	do.	do.	7,450	Sept. 12	0.77	9.3
Do.	Spruce-fir	Northern aspect (under shade).	8,500	do.	0.57	6.9
Do.	do.	Eastern aspect (open)	9,000	Sept. 9	0.78	9.4
Do.	Chaparral	Southern aspect	7,550	Dec. 6	2.00	24.0
Do.	Aspen-fir	Ridge-top	9,000	Dec. 7	1.94	23.3
Beaver Creek	do.	Southern aspect	7,400	Sept. 16	1.50	18.0
Taylor Canyon	do.	Northern aspect	5,200	Jan. 4	2.15	25.8
Do.	do.	do.	5,200	Feb. 28	1.67	20.1
<i>Amelanchier alnifolia</i> Nutt.						
Big Cottonwood Canyon.	Aspen-fir	Northern aspect	7,450	July 12	1.42	17.1
Do.	Chaparral	Southern aspect	7,550	July 19	2.01	24.2
Do.	do.	do.	7,550	Sept. 6	1.53	18.4
Do.	do.	do.	7,550	Sept. 12	1.74	20.9
Do.	Aspen-fir	Northern aspect	7,450	do.	1.24	14.9
Do.	Spruce-fir	Northern aspect (open).	8,500	do.	1.70	20.4
<i>Cornus stolonifera</i> Michx.						
Big Cottonwood Canyon.	Aspen-fir	Northern aspect	7,500	July 13	1.30	15.6

TABLE VIII.—Freezing point depressions and osmotic pressures of the sap of shrubs classified by species, locality, type, situation, altitude, and date of collection—Con.

[Figures in italics represent averages of two or more determinations]

Species and locality	Type	Situation	Altitude	Date of collection	Depression of freezing point	Osmotic pressure
<i>Dasiphora fruticosa</i> (L.) Rydb.			<i>Feet</i>	<i>1920-1921</i>	<i>Degrees C.</i>	<i>Atmospheres</i>
Beaver Creek.....	Lodgepole pine.....	Very gentle southern aspect.	7,400	Sept. 16	1.70	20.4
<i>Grossularia inermis</i> (Rydb.) Coville and Britton						
Big Cottonwood Canyon.	Aspen-fir.....	Northern aspect.....	7,450	July 19	1.21	14.6
<i>Juniperus communis</i> L.						
Beaver Creek.....	Aspen-fir.....	Very gentle northern aspect.	7,300	Sept. 16	1.67	20.1
Do.....	Lodgepole pine.....	Very gentle eastern aspect.	7,400	do.....	1.26	15.2
Big Cottonwood Canyon.	Spruce-fir.....	Northern aspect.....	10,000	Sept. 9	0.68	8.2
<i>Lepargyrea argentea</i> (Nutt) Greene						
Big Cottonwood Canyon.	Aspen-fir.....	Northern aspect.....	7,450	Sept. 12	1.40	16.8
<i>Lepargyrea canadensis</i> (L.) Greene						
Big Cottonwood Canyon.	Aspen-fir.....	Northern aspect.....	7,450	July 12	1.17	14.1
<i>Lonicera involucrata</i> (Richards.) Banks						
Big Cottonwood Canyon.	Aspen-fir.....	Northern aspect.....	7,450	July 11	1.74	20.9
Do.....	do.....	do.....	7,500	July 13	1.99	23.9
Do.....	do.....	do.....	7,450	Sept. 12	1.49	17.9
Do.....	Spruce-fir.....	do.....	8,800	July 25	1.49	17.9
Do.....	do.....	do.....	8,950	Sept. 9	1.39	16.9
<i>Lonicera utahensis</i> S. Wats.						
Big Cottonwood Canyon.	Aspen-fir.....	Northern aspect.....	7,450	July 22	1.47	17.7
Do.....	do.....	do.....	7,450	Sept. 12	1.28	15.4
Do.....	Spruce-fir.....	do.....	8,950	Sept. 9	1.40	16.8
<i>Opulaster malvaceus</i> (Greene) Kuntze						
Big Cottonwood Canyon.	Aspen-fir.....	Northern aspect.....	7,500	July 13	1.55	18.6
Do.....	do.....	do.....	7,450	Sept. 12	1.53	18.4
Do.....	Spruce-fir.....	Northern aspect (open).	8,500	do.....	1.62	19.5
<i>Ribes lacustre</i> (Pers.) Poir.						
Big Cottonwood Canyon.	Aspen-fir.....	Northern aspect.....	7,450	July 19	1.16	14.0
<i>Ribes montigenum</i> McClatchie						
Big Cottonwood Canyon.	Spruce-fir.....	Open ridge top.....	10,000	July 15	1.44	17.3
<i>Ribes viscosissimum</i> Pursh.						
Big Cottonwood Canyon.	Aspen-fir.....	Northern aspect.....	7,450	July 12	1.40	16.8

TABLE VIII.—Freezing point depressions and osmotic pressures of the sap of shrubs classified by species, locality, type, situation, altitude, and date of collection—Con.

[Figures in italics represent averages of two or more determinations]

Species and locality	Type	Situation	Altitude	Date of collection	Depression of freezing point	Osmotic pressure
<i>Ribes wolfii</i> Rothrock					<i>Degrees C.</i>	<i>Atmospheres</i>
Big Cottonwood Canyon.	Aspen-fir.....	Northern aspect.....	<i>Feet</i> 7,450	1920-1921 July 19	1.27	15.3
Do.....	Spruce-fir.....	Northern aspect..... (open).	10,000	July 15	1.36	16.4
<i>Rosa fendleri</i> Crepin						
Big Cottonwood Canyon.	Aspen-fir.....	Northern aspect.....	7,500	July 13	1.30	15.6
Do.....	do.....	Southern aspect.....	7,450	July 19	1.31	15.8
Do.....	do.....	do.....	7,450	July 26	1.48	17.8
<i>Rubus parviflorus</i> Nutt						
Big Cottonwood Canyon.	Aspen-fir.....	Northern aspect.....	7,450	July 12	1.12	13.5
Do.....	Spruce-fir.....	Eastern aspect.....	9,000	Sept. 9	1.14	13.7
<i>Rubus leucodermis</i> Dougl.						
Big Cottonwood Canyon.	Aspen-fir.....	Northern aspect.....	7,500	Sept. 13	0.79	9.5
<i>Salix scouleriana</i> Barratt						
Big Cottonwood Canyon.	Aspen-fir.....	Northern aspect.....	7,500	July 13	1.61	19.4
<i>Salix subcaerulea</i> Piper						
Big Cottonwood Canyon.	Spruce-fir.....	Northern aspect.....	8,950	Sept. 9	1.46	17.6
<i>Sambucus melanocarpa</i> A. Gray						
Big Cottonwood Canyon.	Aspen-fir.....	Northern aspect.....	7,500	July 13	1.13	13.6
Do.....	Spruce-fir.....	Ridge-top.....	10,000	July 15	1.12	13.5
Do.....	Aspen-fir.....	Northern aspect.....	7,500	Sept. 13	1.05	12.6
<i>Sambucus coerulea</i> Raf. (= <i>S. glauca</i> Nutt.)						
Big Cottonwood Canyon.	Aspen-fir.....	Southern aspect.....	7,450	July 26	1.36	16.4
Do.....	do.....	do.....	7,450	Sept. 7	1.26	15.2
Do.....	do.....	Northern aspect.....	7,450	Sept. 12	1.28	15.4
Do.....	Chaparral.....	Southern aspect.....	7,550	do.....	1.46	17.6
<i>Sambucus microbotrys</i> Rydb.						
Big Cottonwood Canyon.	Spruce-fir.....	Northern aspect.....	8,850	Sept. 9	1.15	13.8
<i>Sorbus scopulina</i> Greene						
Big Cottonwood Canyon.	Aspen-fir.....	Northern aspect.....	7,500	July 13	1.84	22.1
<i>Symphoricarpos albus</i> (L.) Blake (= <i>S. racemosus</i> Michx.)						
Big Cottonwood Canyon.	Aspen-fir.....	Northern aspect.....	7,500	July 13	1.91	23.0
<i>Symphoricarpos vaccinioides</i> Rydb.						
Big Cottonwood Canyon.	Aspen-fir.....	Southern aspect.....	7,450	July 19	1.70	20.4
Do.....	do.....	do.....	7,450	Sept. 7	1.67	20.1

TABLE VIII.—Freezing point depressions and osmotic pressures of the sap of shrubs classified by species, locality, type, situation, altitude, and date of collection—Con.

[Figures in italics represent averages of two or more determinations]

Species and locality	Type	Situation	Altitude	Date of collection	Depression of freezing point	Osmotic pressure
<i>Symphoricarpos oreophilus</i> A. Gray			<i>Feet</i>	<i>1920-1921</i>	<i>Degrees C.</i>	<i>Atmospheres</i>
Big Cottonwood Canyon.	Aspen-fir.....	Northern aspect.....	7,450	July 22	1.44	17.3
Do.....	do.....	do.....	7,700	Sept. 9	1.34	16.1
Do.....	Spruce-fir.....	Eastern aspect (open)	9,000	do.....	2.05	24.6
Do.....	do.....	Ridge-top.....	10,000	July 15	1.67	20.1
<i>Arctostaphylos uva-ursi</i> (L.) Spreng.						
Big Cottonwood Canyon.	Spruce-fir.....	do.....	10,000	Sept. 9	0.68	8.2
<i>Taxus canadensis</i> Marshall						
Ogden, Utah.....	.....	City park <sup>a</sup> .....	4,350	Jan. 25	0.85	10.2

<sup>a</sup> Exotic to this site, planted.

TABLE IX.—Freezing point depressions and osmotic pressures of the sap of herbaceous plants, including grasses and a moss on Big Cottonwood Canyon watershed, classified by species, type, situation, altitude, and date of collection

[Figures in italics represent averages of two or more determinations]

Species and type	Situation	Altitude	Date of collection	Depression of freezing point	Osmotic pressure
<i>Achillea lanulosa</i> Nutt.		<i>Feet</i>	<i>1920</i>	<i>Degrees C.</i>	<i>Atmospheres</i>
Aspen-fir.....	Northern aspect.....	7,450	Sept. 10	0.86	10.4
<i>Aconitum bakeri</i> Greene					
Aspen-fir.....	Northern aspect.....	7,450	July 26	0.90	10.8
<i>Actaea arguta</i> Nutt.					
Spruce-fir.....	Northern aspect.....	9,000	Sept. 9	1.04	12.5
<i>Agastache urticifolia</i> (Benth.) Kuntze					
Spruce-fir.....	Northern aspect.....	10,000	July 15	1.16	14.0
Do.....	Northern aspect (open).....	8,850	Sept. 9	1.38	16.6
<i>Amblystegium varium</i> (Hedw.) Lindb.					
Stream bank.....	Edge of water.....	9,000	July 27	0.22	2.7
Do.....	do.....	7,400	Sept. 21	0.42	5.1
<i>Apocynum androsaemifolium</i> L.					
Chaparral.....	Southern aspect.....	6,400	July 21	1.71	20.6
Aspen-fir.....	do.....	7,450	July 26	1.41	17.0
<i>Aquilegia caerulea albiflora</i> A. Gray					
Spruce-fir.....	Ridge-top.....	10,000	July 15	1.40	16.8
<i>Artemisia gnaphaloides</i> Nutt.					
Spruce-fir.....	Ridge-top.....	10,000	July 15	1.06	12.8
Chaparral.....	Southern exposure.....	6,400	Sept. 22	2.04	24.5
Spruce-fir.....	Northern aspect.....	8,900	Sept. 9	0.96	11.6



TABLE IX.—Freezing point depressions and osmotic pressures of the sap of herbaceous plants, including grasses and a moss on Big Cottonwood Canyon watershed, classified by species, type, situation, altitude, and date of collection—Con.

[Figures in italics represent averages of two or more determinations]

Species and type	Situation	Altitude	Date of collection	Depression of freezing point	Osmotic pressure
<i>Aster eatonii</i> (A. Gray) Howell		<i>Feet</i>	<i>1920</i>	<i>Degrees C.</i>	<i>Atmospheres</i>
Aspen-fir.....	Northern aspect.....	7,450	Sept. 10	0.93	11.2
<i>Aster adscendens</i> Lindl.					
Spruce-fir.....	Eastern aspect.....	9,000	Sept. 9	0.82	9.9
<i>Aster engelmannii</i> A. Gray (= <i>Eucephalus engelmannii</i> (A. Gray) Greene)					
Spruce-fir.....	Eastern aspect.....	8,950	do.	1.43	17.3
<i>Aster glaucus</i> T. & G. (= <i>Eucephalus glaucus</i> Nutt.)					
Chaparral.....	Southern aspect.....	7,550	July 26	1.42	17.1
Aspen-fir.....	do.....	7,450	Sept. 7	1.29	15.5
<i>Atragene columbiana</i> Nutt. ( <i>Clematis columbiana</i> (Nutt.) Torr. & Gray)					
Aspen-fir.....	Northern aspect.....	7,450	July 12	1.37	16.5
<i>Balsamorhiza sagittata</i> (Pursh) Nutt.					
Chaparral.....	Southern aspect.....	7,550	July 16	1.73	20.8
Do.....	do.....	7,550	Sept. 6	1.76	21.2
<i>Bromus richardsonii</i> Link.					
Spruce-fir.....	Northern aspect.....	8,950	Sept. 12	1.14	13.7
<i>Caltha leptosepala</i> Hook.					
Spruce-fir.....	Northern aspect.....	9,500	July 15	0.99	11.9
<i>Castilleja lancifolia</i> Rydb.					
Aspen-fir.....	Northern aspect.....	7,500	July 13	1.71	20.6
<i>Castilleja linariaefolia</i> Benth.					
Chaparral.....	Southern aspect.....	7,500	July 19	1.91	23.0
Do.....	do.....	7,500	Sept. 6	1.83	22.0
Do.....	do.....	7,500	Sept. 7	2.23	26.8
<i>Castilleja miniata</i> Dougl.					
Aspen-fir.....	Northern aspect.....	7,450	July 22	1.55	18.6
<i>Castilleja rhexifolia</i> Rydb.					
Spruce-fir.....	Northern aspect.....	10,000	July 15	1.65	19.8
Do.....	Eastern aspect.....	8,950	Sept. 9	1.89	22.7
<i>Chamaenerion angustifolium</i> (L.) Scop.					
Aspen-fir.....	Northern aspect.....	7,450	July 17	0.80	9.6
Spruce-fir.....	Eastern aspect.....	8,950	Sept. 9	0.91	11.0
<i>Chrysopsis fulcrata</i> Greene					
Spruce-fir.....	Eastern aspect.....	9,000	do.	0.99	11.9
<i>Cirsium undulatum</i> (Nutt.) Spreng.					
Aspen-fir.....	Lower southern aspect.....	7,450	Sept. 7	1.24	14.9
<i>Drymocallis glandulosa</i> (Nutt.) Rydb.					
Aspen-fir.....	Northern aspect.....	7,450	July 22	1.19	14.3
Spruce-fir.....	do.....	9,000	July 25	0.77	9.3
Do.....	Northern aspect (open).....	10,000	July 15	1.31	15.8
Aspen-fir.....	Lower southern aspect.....	7,450	Sept. 6	0.85	10.2
Spruce-fir.....	Eastern aspect, (open).....	8,850	Sept. 9	1.45	17.4

TABLE IX.—Freezing point depressions and osmotic pressures of the sap of herbaceous plants, including grasses and a moss on Big Cottonwood Canyon watershed, classified by species, type, situation, altitude, and date of collection—Con.

[Figures in italics represent averages of two or more determinations]

Species and type	Situation	Altitude	Date of collection	Depression of freezing point	Osmotic pressure
<i>Elephantella groenlandica</i> (Retz.) Rydb. (= <i>Pedicularis groenlandica</i> Retz.)		<i>Feet</i>	<i>1920</i>	<i>Degrees C.</i>	<i>Atmospheres</i>
Spruce-fir.....	Northern aspect.....	8,800	July 25	1.15	13.8
<i>Equisetum laevigatum</i> A. Br.					
Chaparral.....	Southern aspect.....	6,400	July 21	1.25	15.0
<i>Erigeron speciosus</i> (Lindl.) DC.					
Spruce-fir.....	Northern aspect.....	8,950	Sept. 9	0.99	11.9
<i>Eriogonum heracleoides</i> Nutt.					
Spruce-fir.....	Eastern aspect.....	9,000	do	1.04	12.5
<i>Eriogonum neglectum</i> Greene					
Chaparral.....	Southern aspect.....	7,500	July 19	1.80	21.6
Do.....	do.....	7,500	Sept. 6	1.46	17.6
<i>Festuca confinis</i> Vasey					
Chaparral.....	Southern aspect.....	7,500	Sept. 12	1.58	19.0
<i>Fragaria platypetala</i> Rydb.					
Aspen-fir.....	Northern aspect.....	7,450	July 22	1.11	13.4
Do.....	Northern aspect, under aspen.	7,450	Sept. 13	0.49	5.9
<i>Frasera speciosa</i> Dougl.					
Aspen-fir.....	Northern aspect.....	7,450	July 22	1.12	13.5
<i>Geranium viscosissimum</i> F. & M.					
Aspen-fir.....	Lower southern aspect.....	7,450	July 26	1.16	14.0
Do.....	Northern aspect.....	7,500	July 13	1.04	12.5
Do.....	do.....	7,500	Sept. 13	0.72	8.7
<i>Gilia aggregata</i> (Pursh) Spreng.					
Spruce-fir.....	Northern aspect, open.....	8,850	Sept. 12	1.26	15.2
<i>Heracleum lanatum</i> Michx.					
Aspen-fir.....	Northern aspect.....	7,450	July 19	1.00	12.0
<i>Humulus lupulus neomexicanus</i> Nels. & Cockerell					
Aspen-fir.....	Northern aspect.....	7,450	July 27	0.85	10.2
<i>Ivesia gordonii</i> (Hook) T. & G. (= <i>Horkelia gordonii</i> Hook.)					
Spruce-fir.....	Eastern aspect, open.....	9,000	Sept. 9	0.89	10.7
<i>Lappula floribunda</i> (Lehm.) Greene					
Aspen-fir.....	Northern aspect.....	7,450	July 27	0.70	8.4
Spruce-fir.....	Ridge-top.....	10,000	July 15	0.81	9.8
<i>Lathyrus coriaceus</i> White					
Spruce-fir.....	Northern aspect, open.....	10,000	July 15	0.93	11.2
<i>Linum lewisii</i> Pursh.					
Spruce-fir.....	Eastern aspect, open.....	9,000	Sept. 9	1.40	16.8
<i>Lithospermum ruderale</i> Dougl. (= <i>L. pilosum</i> Nutt.)					
Aspen-fir.....	Lower southern aspect.....	7,450	Sept. 6	1.18	14.2

TABLE IX.—Freezing point depressions and osmotic pressures of the sap of herbaceous plants, including grasses and a moss on Big Cottonwood Canyon watershed, classified by species, type, situation, altitude, and date of collection—Con.

[Figures in italics represent averages of two or more determinations]

Species and type	Situation	Altitude	Date of collection	Depression of freezing point	Osmotic pressure
<i>Lupinus alpestris</i> A. Nels.				<i>Degrees C.</i>	<i>Atmospheres</i>
Spruce-fir.....	Northern aspect.....	10,000	1920 July 15	0.90	10.8
Do.....	Eastern aspect.....	8,900	Sept. 9	1.09	13.1
<i>Machaeranthera viscosa</i> (Nutt.) Greene					
Chaparral.....	Southern aspect.....	7,500	Sept. 7	1.86	22.4
<i>Macronema watsoni</i> (A. Gray) Greene					
Chaparral.....	Southern aspect.....	6,400	Sept. 22	1.72	20.7
<i>Madronella oblongifolia</i> Rydb.					
Spruce-fir.....	Ridge-top.....	10,000	July 15	1.15	13.8
Do.....	Eastern exposure, open.....	9,000	Sept. 9	1.02	12.3
<i>Mertensia sampsonii</i> Tidestrom					
Aspen-fir.....	Northern aspect.....	7,500	July 13	1.00	12.0
Spruce-fir.....	Ridge-top.....	10,000	July 15	0.83	10.0
Do.....	Eastern aspect, open.....	9,000	Sept. 9	0.97	11.7
<i>Mimulus guttatus</i> DC.					
Spruce-fir.....	Wet meadow.....	8,800	July 25	0.58	7.0
<i>Orthocarpus tolmiei</i> H. & A.					
Aspen-fir.....	Lower southern aspect.....	7,450	Sept. 7	1.87	22.5
Spruce-fir.....	Northern aspect, open.....	8,850	Sept. 9	1.76	21.2
<i>Pentstemon cyananthus</i> Hook.					
Spruce-fir.....	Northern aspect, open.....	8,800	July 25	1.40	16.8
<i>Pentstemon humilis</i> Nutt.					
Chaparral.....	Southern aspect.....	7,500	July 26	2.05	24.6
<i>Polemonium occidentale</i> Greene					
Aspen-fir.....	Northern aspect.....	7,500	July 19	1.15	13.8
<i>Potentilla filipes</i> Rydb.					
Aspen-fir.....	Northern aspect.....	7,500	July 13	1.21	14.6
<i>Potentilla nuttallii</i> Lehm.					
Spruce-fir.....	Northern aspect, wet ground.	8,950	Sept. 9	0.87	10.5
<i>Ranunculus maximus</i> Greene					
Spruce-fir.....	Northern aspect.....	8,800	July 25	1.05	12.6
<i>Rudbeckia occidentalis</i> Nutt.					
Aspen-fir.....	Northern aspect.....	7,450	July 19	0.91	11.0
Spruce-fir.....	Eastern aspect.....	8,900	Sept. 9	0.91	11.0
<i>Sedum stenopetalum</i> Pursh.					
Aspen-fir.....	Southern aspect.....	7,450	July 26	0.53	6.4
Do.....	do.....	7,450	Sept. 7	0.70	8.4
<i>Senecio serra</i> Hook.					
Aspen-fir.....	Northern aspect.....	7,450	July 26	0.92	11.1
<i>Solidago trinerrata</i> Greene					
Chaparral.....	Southern aspect.....	6,400	Sept. 22	2.27	27.3

TABLE IX.—Freezing point depressions and osmotic pressures of the sap of herbaceous plants, including grasses and a moss on Big Cottonwood Canyon watershed, classified by species, type, situation, altitude, and date of collection—Con.

[Figures in italics represent averages of two or more determinations]

Species and type	Situation	Altitude	Date of collection	Depression of freezing point	Osmotic pressure
<i>Stipa nelsonii</i> Scribner				<i>Degrees C.</i>	<i>Atmospheres</i>
Spruce-fir.....	Northern aspect, open.....	<i>Feet</i> 8,850	<i>1920</i> Sept. 12	1.33	16.0
<i>Thalictrum fendleri</i> Engelm.					
Aspen-fir.....	Northern aspect.....	7,450	July 17	1.51	18.2
Spruce-fir.....	Ridge-top.....	10,000	July 15	1.43	17.2
Do.....	Eastern aspect, open.....	8,950	Sept. 9	1.41	17.0
<i>Tithymalus robustus</i> (Engelm.) Small (= <i>Euphorbia robusta</i> (Engelm.) Small)					
Chaparral.....	Southern aspect.....	7,500	July 26	1.56	18.8
<i>Urtica gracilis</i> Ait.					
Aspen-fir.....	Northern aspect.....	7,450	July 23	0.88	10.6
<i>Vagnera liliacea</i> (Greene) Rydb.					
Aspen-fir.....	Northern aspect.....	7,450	July 27	0.98	11.8
<i>Valeriana ceratophylla</i> (Hook.) Piper					
Spruce-fir.....	Northern aspect.....	8,800	July 25	0.97	11.7
<i>Veratrum speciosum</i> Rydb.					
Spruce-fir.....	Northern aspect.....	8,800	July 25	0.97	11.7
<i>Viguiera multiflora</i> (Nutt.) Blake (= <i>Gymnolomia multiflora</i> (Nutt.) Benth. & Hook.)					
Chaparral.....	Southern aspect.....	7,500	Sept. 7	1.57	18.9
Spruce-fir.....	Northern aspect.....	8,950	Sept. 9	1.02	12.3
<i>Zauschneria garretti</i> A. Nels.					
Chaparral.....	Southern aspect.....	6,400	Sept. 22	1.16	14.0

TABLE X.—Summary of sap concentrations of all species by type, growth form, and season, Wasatch Mountain series

Type and growth form.	Season.	Depression of freezing point.	Osmotic pressure.
		<i>Degrees C.</i>	<i>Atmospheres</i>
Stream bank:			
Trees.....	Summer.....	1.36	16.4
Shrubs.....	do.....	1.60	19.2
Moss.....	do.....	0.22	2.7
Do.....	Autumn.....	0.42	5.1
Sagebrush:			
Shrubs.....	Summer.....	2.51	30.1
Do.....	Autumn.....	2.38	28.6
Do.....	Winter.....	2.75	33.0
Do.....	Spring.....	2.35	28.2
Juniper:			
Trees.....	Summer.....	2.19	26.3
Do.....	Autumn.....	1.32	15.9
Do.....	Winter.....	2.26	27.2
Shrubs.....	Summer.....	1.86	22.4
Do.....	Autumn.....	1.90	22.8
Western yellow pine:			
Trees.....	Summer.....	1.85	22.2
Do.....	Autumn.....	1.45	17.4
Do.....	Winter.....	1.41	17.0
Shrubs.....	Summer.....	2.07	24.9
Do.....	Autumn.....	1.68	20.2

TABLE X.—Summary of sap concentrations of all species by type, growth, form, and season, Wasatch Mountain series—Continued

Type and growth form.	Season.	Depression of freezing point.	Osmotic pressure.
		<i>Degrees C.</i>	<i>Atmospheres</i>
Chaparral:			
Trees.....	Summer.....	1.60	19.2
Do.....	Autumn.....	0.93	11.2
Do.....	Winter.....	2.17	26.1
Shrubs.....	Summer.....	1.93	23.2
Do.....	Autumn.....	1.77	21.3
Do.....	Winter.....	2.66	31.9
Herbs.....	Summer.....	1.68	20.2
Do.....	Autumn.....	1.77	21.3
Aspen-fir:			
Trees.....	Summer.....	1.39	16.7
Do.....	Autumn.....	1.23	14.8
Do.....	Winter.....	1.06	12.8
Shrubs.....	Summer.....	1.42	17.1
Do.....	Autumn.....	1.32	15.9
Do.....	Winter.....	2.20	26.4
Herbs.....	Summer.....	1.08	13.0
Do.....	Autumn.....	0.99	11.9
Spruce-fir:			
Trees.....	Summer.....	1.56	18.8
Do.....	Autumn.....	1.10	13.2
Do.....	Winter.....	0.81	9.8
Shrubs.....	Summer.....	1.50	18.0
Do.....	Autumn.....	1.29	15.5
Herbs.....	Summer.....	1.05	12.6
Do.....	Autumn.....	1.15	13.8

TABLE XI.—Freezing point depressions and osmotic pressures of the sap of trees on Ephraim Canyon watershed, central Utah, classified by species, type, situation, altitude, and date of collection

[Figures in italics represent averages of two or more determinations]

Species and type	Situation	Altitude	Date of collection	Depression of freezing point	Osmotic pressure
		<i>Feet</i>	<i>1921</i>	<i>Degrees C.</i>	<i>Atmospheres</i>
<i>Pinus edulis</i> Engelm.					
Pinon-juniper.....	Outwash from canyon.....	5,800	July 21	1.86	22.4
Do.....	Southern aspect.....	6,000	July 23	1.72	20.7
Do.....	do.....	7,400	July 19	1.32	15.9
Do.....	do.....	7,400	July 26	1.70	20.4
<i>Juniperus utahensis</i> (Engelm.) Lemm.					
Pinon-juniper.....	Outwash from canyon.....	5,800	July 21	1.90	22.8
Do.....	Southern aspect.....	6,000	July 23	1.33	16.0
Oak brush.....	do.....	7,200	July 26	1.27	15.3
<i>Juniperus scopulorum</i> Sarg.					
Oak brush.....	Northern aspect.....	7,400	July 19	1.42	17.1
<i>Pinus ponderosa scopulorum</i> Engelm.					
Oak brush.....	Sagebrush flat <sup>b</sup> .....	7,600	July 19	1.91	23.0
Do.....	do.....	7,400	do.....	1.68	20.2
Do.....	Southern aspect <sup>a</sup> .....	7,400	do.....	1.54	18.5
<i>Pinus ponderosa</i> Laws.					
Oak brush.....	Northern aspect <sup>a</sup> .....	7,400	July 19	1.47	17.7
Do.....	Sagebrush flat <sup>a</sup> .....	7,400	do.....	1.65	19.8

<sup>a</sup> Exotic to this site, planted.<sup>b</sup> Planted, although native to site.

TABLE XI.—Freezing point depressions and osmotic pressures of the sap of trees on Ephraim Canyon watershed, central Utah, classified by species, type, situation, altitude, and date of collection—Continued

[Figures in italics represent averages of two or more determinations]

Species and type	Situation	Altitude	Date of collection	Depression of freezing point	Osmotic pressure
<i>Pseudotsuga taxifolia</i> (Lam.) Britton		<i>Feet</i>	<i>1921</i>	<i>Degrees C.</i>	<i>Atmospheres</i>
Oak brush.....	Northern aspect <sup>b</sup> .....	7,400	July 19	1.65	19.8
Do.....	do.....	7,400	July 26	1.24	14.9
Aspen-fir.....	Western aspect.....	8,800	July 14	1.92	23.1
Do.....	do.....	8,800	July 22	1.66	20.0
Do.....	Northern aspect.....	8,800	July 24	1.39	16.7
<i>Abies concolor</i> (Gord.) Parry					
Oak brush.....	Northern aspect.....	7,400	July 26	1.55	18.6
Aspen-fir.....	Western aspect.....	8,800	July 14	1.65	19.8
Do.....	Northern aspect.....	8,800	July 24	1.20	14.4
<i>Picea parryana</i> (André) Parry					
Oak brush.....	Northern aspect.....	7,400	July 26	1.04	12.5
Aspen-fir.....	Western aspect.....	8,700	July 14	1.43	17.2
Do.....	Northern aspect.....	8,800	July 24	1.08	13.0
<i>Pinus flexilis</i> James					
Aspen-fir.....	Northern aspect.....	8,800	July 24	1.10	13.2
Spruce-fir.....	Southern aspect.....	10,000	July 18	1.20	14.4
Do.....	do.....	10,000	July 22	1.14	13.7
<i>Picea engelmanni</i> (Parry) Engelm.					
Aspen-fir.....	Western aspect.....	8,800	July 14	1.23	14.8
Do.....	Northern aspect.....	8,800	July 24	0.97	11.7
Spruce-fir.....	do.....	10,000	July 18	1.37	16.5
Do.....	do.....	10,000	July 22	1.17	14.1
Do.....	Southern aspect.....	10,000	July 18	1.16	14.0
Do.....	do.....	10,000	July 22	1.15	13.8
<i>Abies lasiocarpa</i> (Hook.) Nutt.					
Oak brush.....	Northern aspect.....	7,500	July 26	1.24	14.9
Aspen-fir.....	Western aspect.....	8,700	July 14	1.37	16.5
Do.....	Northern aspect.....	8,800	July 24	1.01	12.2
Spruce-fir.....	do.....	10,000	July 15	1.19	14.3
Do.....	do.....	10,000	July 18	0.65	7.8
Do.....	do.....	10,000	July 22	0.78	9.4
Do.....	Southern aspect.....	10,000	July 18	1.36	16.4
Do.....	do.....	10,000	July 22	0.98	11.8
<i>Acer grandidentatum</i> Nutt.					
Oak brush.....	Northern aspect.....	7,400	July 19	1.38	16.6
<i>Acer glabrum</i> Torr.					
Aspen-fir.....	Northern aspect.....	8,800	July 24	1.07	12.9
<i>Populus tremuloides</i> Michx.					
Oak brush.....	Northern aspect.....	7,800	July 26	1.96	23.6
Aspen-fir.....	Western aspect.....	8,800	July 14	2.29	27.5
Spruce-fir.....	Southern aspect.....	9,500	do.....	2.17	26.1
Do.....	do.....	9,500	July 18	2.19	26.3

<sup>b</sup> Planted, although native to site.

TABLE XII.—Freezing point depressions and osmotic pressures of the sap of shrubs on Ephraim Canyon watershed, central Utah, classified by species, type, situation, altitude, and date of collection

[Figures in italics represent averages of two or more determinations]

Species and type	Situation	Altitude	Date of collection	Depression of freezing point	Osmotic pressure
<i>Atriplex confertifolia</i> S. Wats.				Degrees C.	Atmospheres
Greasewood-shadscale	Alkali flat	Feet 5,600	1921 July 23	5.14	61.4
Pinon-juniper	Southern aspect	6,200	do	2.62	31.4
<i>Atriplex tridentata</i> O. Kuntze					
Greasewood-shadscale	Alkali flat	5,600	July 23	5.65	67.5
<i>Sarcobatus vermiculatus</i> (Hook.) Torr.					
Greasewood-shadscale	Alkali flat	5,600	July 23	2.52	30.3
Sagebrush	Outwash from canyon	5,800	do	2.23	26.8
<i>Eurotia lanata</i> (Pursh) Moq.					
Greasewood-shadscale	Alkali flat	5,600	July 21	2.98	35.8
Oak brush	Southern aspect	7,400	July 26	1.72	20.7
<i>Artemisia tridentata</i> Nutt.					
Sagebrush	Outwash from canyon	5,800	July 21	2.93	35.2
Do	do	5,800	July 23	2.92	35.0
Oak brush	Southern aspect	7,400	July 26	1.68	20.2
Do	Sagebrush flat	7,400	July 19	1.38	16.6
Spruce-fir	Northern aspect	10,700	July 21	1.21	14.6
<i>Chrysothamnus nauseosus</i> (Pall.) Britt.					
Sagebrush	Outwash from canyon	5,800	July 23	1.32	15.9
Pinon-juniper	Steep southern aspect	6,000	do	1.53	18.4
Oak brush	Sagebrush flat	7,400	July 26	1.68	20.2
<i>Chrysothamnus puberulus</i> (D. C. Eaton) Greene					
Pinon-juniper	Southern aspect	6,000	July 23	1.63	19.6
<i>Chrysothamnus</i> sp.					
Oak brush	Southern aspect	7,400	July 26	2.16	26.0
<i>Chrysothamnus lanceolatus</i> Nutt.					
Spruce-fir	Northern aspect	10,500	July 15	1.37	16.5
Do	do	10,500	July 21	1.37	16.5
<i>Ephedra viridis</i> Coville					
Pinon-juniper	Southern aspect	6,000	July 23	1.23	14.8
<i>Rhus trilobata</i> Nutt.					
Pinon-juniper	Southern aspect	6,000	July 23	1.94	23.3
<i>Peraphyllum ramosissimum</i> Nutt.					
Pinon-juniper	Southern aspect	6,000	July 23	2.18	26.2
Oak brush	do	7,400	July 26	2.08	25.0
<i>Quercus utahensis</i> (DC.) Rydb.					
Pinon-juniper	Southern aspect	6,000	July 23	1.64	19.7
Oak brush	Northern aspect	7,400	July 18	1.67	20.1
Do	Southern aspect	7,400	July 19	1.72	20.7
Do	do	7,400	July 26	1.54	18.5
Aspen-fir	do	8,600	July 25	1.28	15.4
<i>Arctostaphylos pungens</i> H. B. K.					
Oak brush	Southern aspect	7,400	July 19	1.50	18.0
Aspen-fir	do	8,600	July 26	1.43	17.2
<i>Cercocarpus Montanus</i> Raf. Nutt.					
Oak brush	Southern aspect	7,400	July 26	1.31	15.8
Aspen-fir	do	8,600	July 25	1.31	15.8

TABLE XII.—Freezing point depressions and osmotic pressures of the sap of shrubs on Ephraim Canyon watershed, central Utah, classified by species, type, situation, altitude, and date of collection—Continued

[Figures in italics represent averages of two or more determinations]

Species and type	Situation	Altitude	Date of collection	Depression of freezing point	Osmotic pressure
<i>Cercocarpus ledifolius</i> Nutt.		<i>Feet</i>	<i>1921</i>	<i>Degrees C.</i>	<i>Atmospheres</i>
Aspen-fir.....	Southern aspect.....	8,600	July 24	1.05	12.6
<i>Ceanothus fendleri</i> A. Gray					
Oak brush.....	Southern aspect.....	7,400	July 26	1.48	17.8
Aspen-fir.....	do.....	8,600	July 25	1.23	14.8
<i>Amelanchier alnifolia</i> Nutt.					
Oak brush.....	Northern aspect.....	7,400	July 19	1.58	19.0
Do.....	Southern aspect.....	7,400	do.....	1.89	22.7
Aspen-fir.....	Western aspect.....	8,600	July 27	1.45	17.4
<i>Prunus melanocarpa</i> (A. Nels.) Rydb.					
Oak brush.....	Northern aspect.....	7,400	July 19	1.50	18.0
Aspen-fir.....	Western aspect.....	8,600	July 27	1.43	17.2
<i>Odostemon repens</i> (Lindl.) Cocker.					
Oak brush.....	Northern aspect.....	7,400	July 19	1.39	16.7
Do.....	Southern aspect.....	7,400	July 26	1.73	20.8
Aspen-fir.....	do.....	8,600	July 25	1.41	17.0
<i>Purshia tridentata</i> (Pursh) D C.					
Oak brush.....	Southern aspect.....	7,400	July 26	1.39	16.7
<i>Pachystima myrsinites</i> (Pursh) Raf.					
Oak brush.....	Southern aspect.....	7,400	July 26	1.13	13.6
Aspen-fir.....	do.....	8,600	July 25	0.97	11.7
<i>Rosa fendleri</i> Crepin.					
Oak brush.....	Northern aspect.....	7,400	July 19	1.43	17.2
Aspen-fir.....	Southern aspect.....	8,600	July 25	1.20	14.4
<i>Symphoricarpos oreophilus</i> A. Gray					
Oak brush.....	Northern aspect.....	7,400	July 19	2.02	24.3
Do.....	Southern aspect.....	7,400	July 26	2.27	27.3
Aspen-fir.....	do.....	8,600	July 14	2.07	24.9
Spruce-fir.....	Northern aspect.....	10,000	July 22	1.76	21.2
Do.....	Southern aspect.....	9,800	do.....	1.98	23.8
<i>Juniperus communis</i> L.					
Oak brush.....	Northern aspect.....	7,400	July 26	1.01	12.2
Aspen-fir.....	Southern aspect.....	8,600	July 25	0.98	11.8
<i>Lepargyrea canadensis</i> (L.) Greene					
Aspen-fir.....	Western aspect.....	8,600	July 24	1.18	14.2
<i>Lonicera involucrata</i> (Richards.) Banks					
Aspen-fir.....	Western aspect.....	8,600	July 24	1.49	17.9
<i>Lonicera utahensis</i> S. Wats.					
Aspen-fir.....	Western aspect.....	8,600	July 25	1.23	14.8
<i>Grossularia inermis</i> (Rydb.) Coville and Britton					
Spruce-fir.....	Northern aspect.....	10,000	July 18	1.39	16.7
Do.....	Southern aspect.....	10,000	July 12	1.69	20.3
Do.....	do.....	10,000	July 15	1.59	19.1
<i>Sambucus microbotrys</i> Rydb.					
Spruce-fir.....	Northern aspect.....	10,000	July 18	1.08	13.0
Do.....	Southern aspect.....	10,000	July 12	1.14	13.7
<i>Ribes cereum</i> Dougl.					
Spruce-fir.....	Southern aspect.....	10,000	July 12	1.18	14.2



TABLE XIII.—Freezing point depressions and osmotic pressures of the sap of herbs on Ephraim Canyon watershed, central Utah, classified by species, type, situation, altitude, and date of collection

[Figures in italics represent averages of two or more determinations]

Species and type	Situation	Altitude	Date of collection	Depression of freezing point	Osmotic pressure
<i>Achillea lanulosa</i> Nutt.		<i>Feet</i>	<i>1921</i>	<i>Degrees C.</i>	<i>Atmospheres</i>
Oak brush.....	Northern aspect.....	7,400	July 19	0.75	9.0
Do.....	Sagebrush flat (under sage).....	7,400	July 26	1.32	15.9
Do.....	Sagebrush flat (in open).....	7,400	do.....	1.49	17.9
Spruce-fir.....	Southern aspect.....	9,800	July 12	1.36	16.4
<i>Aconitum columbianum</i> Nutt.					
Spruce-fir.....	Northern aspect.....	10,000	July 18	0.88	10.6
<i>Agoseris agrestis</i> Osterh.					
Spruce-fir.....	Southern aspect.....	10,000	July 18	0.72	8.7
<i>Agoseris</i> sp.					
Spruce-fir.....	Southern aspect.....	10,000	July 18	0.92	11.1
<i>Agropyron dasystachyum</i> (Hook.) Scribn.					
Spruce-fir.....	Southern aspect.....	10,000	July 15	1.67	20.1
<i>Agropyron riparium</i> S. & S.					
Oak brush.....	Sagebrush flat.....	7,400	July 26	1.19	14.3
<i>Agropyron violaceum</i> (Hornem.) Lange					
Spruce-fir.....	Southern aspect.....	10,000	July 14	1.41	17.0
<i>Alsine jamesiana</i> (Torr.) Heller					
Oak brush.....	Northern aspect.....	7,400	July 26	1.21	14.6
Spruce-fir.....	Southern aspect.....	10,000	July 12	1.20	14.4
<i>Aquilegia caerulea</i> James					
Aspen-fir.....	Gentle northern aspect.....	8,700	July 26	1.13	13.6
Spruce-fir.....	Northern aspect.....	10,000	July 18	1.00	12.0
Do.....	Southern aspect.....	10,000	July 12	1.61	19.4
<i>Arnica cordifolia</i> Hook.					
Spruce-fir.....	Northern aspect.....	10,000	July 15	0.76	9.2
<i>Artemisia discolor</i> Dougl.					
Spruce-fir.....	Southern aspect.....	10,200	July 12	0.92	11.1
<i>Astragalus decumbens</i> (Nutt.) A. Gray					
Spruce-fir.....	Southern aspect.....	10,000	July 14	1.12	13.5
<i>Bromus marginatus</i> Nees.					
Spruce-fir.....	Southern aspect.....	10,000	July 12	1.27	15.3
<i>Caltha rotundifolia</i> (Huth) Greene					
Spruce-fir.....	Southern aspect.....	10,000	July 12	1.14	13.7
<i>Cardamine cordifolia</i> A. Gray					
Spruce-fir.....	Northern aspect.....	10,000	July 15	0.67	8.1
<i>Castilleja flava</i> S. Wats.					
Spruce-fir.....	Southern aspect.....	10,000	July 14	1.56	18.8
<i>Castilleja rhexifolia</i> Rydb.					
Spruce-fir.....	Northern aspect.....	10,000	July, 18	1.34	16.1

TABLE XIII.—Freezing point depressions and osmotic pressures of the sap of herbs on Ephraim Canyon watershed, central Utah, classified by species, type, situation, altitude, and date of collection—Continued

[Figures in italics represent averages of two or more determinations]

Species and type	Situation	Altitude	Date of collection	Depression of freezing point	Osmotic pressure
<i>Chamaenerion angustifolium</i> (L.) Scop.				<i>Degrees C.</i>	<i>Atmospheres</i>
Spruce-fir.....	Northern aspect.....	<i>Feet</i> 10,000	1921 July 18	0.90	10.8
<i>Chenopodium album</i> L.					
Aspen-fir.....	Northern aspect.....	8,700	July 25	1.17	14.1
<i>Claytonia lanceolata</i> Pursh					
Spruce-fir.....	Northern aspect (wet).....	10,000	July 18	0.88	4.6
<i>Cogswellia simplex</i> (S. Wats.) Rydb.					
Spruce-fir.....	Southern aspect.....	10,000	July 18	1.31	15.8
<i>Collomia linearis</i> Nutt.					
Aspen-fir.....	Northern aspect.....	8,700	July 25	0.92	11.1
<i>Comandra pallida</i> A. DC.					
Oak brush.....	Northern aspect.....	7,400	July 26	1.62	19.5
Aspen-fir.....	Southern aspect.....	8,600	July 25	1.49	17.9
<i>Delphinium barbeyi</i> Huth					
Spruce-fir.....	Northern aspect.....	10,000	July 18	1.00	12.0
Do.....	Southern aspect.....	10,000	July 12	1.27	15.3
<i>Delphinium menziesii</i> DC.					
Spruce-fir.....	Southern aspect.....	10,000	July 12	1.65	19.8
<i>Dodecatheon pauciflorum</i> (Durand) Greene					
Spruce-fir.....	Northern aspect (wet).....	10,000	July 15	0.71	8.6
<i>Drymocallis glandulosa</i> (Lindl.) Rydb.					
Spruce-fir.....	Northern aspect.....	10,000	July 18	1.19	14.3
<i>Equisetum arvense</i> L.					
Aspen-fir.....	Western aspect (wet).....	8,700	July 24	0.82	9.9
<i>Eriogonum</i> sp.					
Spruce-fir.....	Southern aspect.....	10,000	July 18	0.97	11.7
<i>Cheirinia aspera</i> (Nutt.) Rydb.					
Spruce-fir.....	Southern aspect.....	10,000	do.....	0.76	9.2
<i>Erythronium grandiflorum</i> Pursh					
Spruce-fir.....	Northern aspect.....	10,000	July 22	1.03	12.4
Do.....	Southern aspect.....	10,000	July 12	1.09	13.1
<i>Fragaria bracteata</i> Heller					
Spruce-fir.....	Northern aspect.....	10,000	July 15	1.20	14.4
<i>Frasera speciosa</i> Griseb.					
Oak brush.....	Northern aspect.....	7,400	July 19	1.26	15.2
Aspen-fir.....	do.....	8,700	July 14	1.07	12.9
<i>Galium boreale</i> L.					
Oak brush.....	Northern aspect.....	7,400	July 19	1.19	14.3
<i>Geranium viscosissimum</i> F. & M.					
Oak brush.....	Northern aspect.....	7,400	July 19	1.08	13.0
Aspen-fir.....	do.....	8,700	July 14	0.95	11.4
Spruce-fir.....	do.....	10,000	July 18	0.86	10.4

TABLE XIII.—Freezing point depressions and osmotic pressures of the sap of herbs on Ephraim Canyon watershed, central Utah, classified by species, type, situation, altitude, and date of collection—Continued

[Figures in italics represent averages of two or more determinations]

Species and type	Situation	Altitude	Date of collection	Depression of freezing point	Osmotic pressure
<i>Gilia micrantha</i> (Kell.) A. Nels.		<i>Feet</i>	<i>1921</i>	<i>Degrees C.</i>	<i>Atmospheres</i>
Spruce-fir.....	Southern aspect.....	10,000	July 12	1.32	15.9
<i>Viguiera multiflora</i> (Nutt.) Blake					
Spruce-fir.....	Southern aspect.....	10,000	July 18	1.04	12.5
<i>Helenium hoopesii</i> A. Gray					
Aspen-fir.....	Northern aspect.....	8,600	July 27	0.99	11.9
<i>Helianthella uniflora</i> (Nutt.) T. & G.					
Spruce-fir.....	Northern aspect.....	10,000	July 21	0.86	10.4
<i>Heracleum lanatum</i> Michx.					
Spruce-fir.....	Northern aspect.....	10,000	July 18	1.23	14.8
<i>Hordeum nodosum</i> L.					
Spruce-fir.....	Northern aspect.....	10,000	July 14	1.41	17.0
<i>Iresia gordonii</i> (Hook.) Torr. & Gray					
Spruce-fir.....	Northern aspect.....	10,000	July 21	1.38	16.6
<i>Hyoscyamus niger</i> L.					
Oak brush.....	Northern aspect.....	7,400	July 23	0.95	11.4
<i>Iva axillaris</i> Pursh					
Aspen-fir.....	Western aspect.....	8,700	July 27	1.12	13.5
<i>Lathyrus leucanthus</i> Rydb.					
Aspen-fir.....	Northern aspect.....	8,700	July 14	1.06	12.8
Spruce-fir.....	do.....	10,000	July 18	0.96	11.6
Do.....	Southern aspect.....	10,000	July 12	1.12	13.5
<i>Lavauria flava</i> A. Nels.					
Spruce-fir.....	Southern aspect.....	10,000	July 18	0.70	8.4
<i>Leontodon tararacum</i> L.					
Oak brush.....	Northern aspect.....	7,400	July 19	1.17	14.1
Do.....	Sagebrush flat (under sage).....	7,400	do.....	1.26	15.2
Do.....	Sagebrush flat (in open).....	7,400	do.....	1.08	13.0
Spruce-fir.....	Southern aspect.....	10,000	July 12	0.96	11.6
<i>Lesquerella utahensis</i> Rydb.					
Spruce-fir.....	Southern aspect.....	10,000	July 14	0.97	11.7
<i>Ligusticum filicinum</i> S. Wats.					
Aspen-fir.....	Northern aspect.....	8,700	July 25	1.14	13.7
Spruce-fir.....	do.....	10,000	July 18	1.21	14.6
Do.....	Southern aspect.....	10,000	do.....	1.32	15.9
<i>Lupinus alpestris</i> A. Nels.					
Aspen-fir.....	Northern aspect.....	8,700	July 14	0.85	10.2
Spruce-fir.....	do.....	10,000	July 18	0.92	11.1
<i>Marrubium vulgare</i> L.					
Pinon-juniper.....	Southern aspect.....	6,000	July 23	1.79	21.5

TABLE XIII.—Freezing point depressions and osmotic pressures of the sap of herbs on Ephraim Canyon watershed, central Utah, classified by species, type, situation, altitude, and date of collection—Continued

[Figures in italics represent averages of two or more determinations]

Species and type	Situation	Altitude	Date of collection	Depression of freezing point	Osmotic pressure
<i>Melica spectabilis</i> Scribn.		<i>Feet</i>	<i>1921</i>	<i>Degrees C.</i>	<i>Atmospheres</i>
Spruce-fir.....	Southern aspect.....	10,000	July 15	1.08	13.0
<i>Mertensia sampsonii</i> Tidestrom					
Spruce-fir.....	Southern aspect.....	10,000	July 14	0.81	9.8
<i>Opuntia polyacantha</i> Haw.					
Oak brush.....	Sagebrush flat.....	7,400	July 19	0.54	6.5
<i>Orthocarpus tolmiei</i> H. & A.					
Spruce-fir.....	Southern aspect.....	10,000	July 14	1.36	16.4
<i>Osmorrhiza obtusa</i> (Coult. & Rose) Fernald					
Spruce-fir.....	Northern aspect.....	10,000	July 18	1.05	12.6
<i>Oxyria digyna</i> (L.) Hill					
Spruce-fir.....	Northern aspect.....	10,000	July 22	0.62	7.5
<i>Paeonia brownii</i> Dougl.					
Aspen-fir.....	Western aspect <i>a</i> .....	8,700	July 25	1.14	13.7
<i>Pentstemon procerus</i> Dougl.					
Spruce-fir.....	Southern aspect.....	10,000	July 12	1.34	16.1
<i>Phacelia sericea</i> (Graham) A. Gray					
Spruce-fir.....	Northern aspect.....	10,000	July 15	1.41	17.0
<i>Plantago tweedyi</i> A. Gray					
Spruce-fir.....	Northern aspect.....	10,000	July 18	0.70	8.4
<i>Poa nevadensis</i> Vasey					
Spruce-fir.....	Southern aspect.....	10,000	July 24	1.50	18.0
<i>Polemonium occidentale</i> Greene					
Aspen-fir.....	Northern aspect.....	8,700	July 14	1.08	13.0
Spruce-fir.....	do.....	10,000	July 18	1.11	13.4
Do.....	Southern aspect.....	10,000	July 12	1.01	12.2
<i>Polygonum douglasii</i> Greene					
Aspen-fir.....	Northern aspect.....	8,700	July 25	0.79	9.5
<i>Potentilla filipes</i> Rydb.					
Spruce-fir.....	Southern aspect.....	10,000	July 12	0.98	11.8
<i>Pseudocymopterus tidestromii</i> Coult. & Rose					
Spruce-fir.....	Southern aspect.....	10,000	July 12	1.32	15.9
<i>Ranunculus inamoenus</i> Greene					
Spruce-fir.....	Northern aspect.....	10,000	July 18	1.16	14.0
Do.....	Southern aspect.....	10,000	July 15	1.46	17.6
<i>Rudbeckia occidentalis</i> Nutt.					
Aspen-fir.....	Northern aspect.....	8,700	July 18	0.99	11.9

*a* Exotic to this site, planted.

TABLE XIII.—Freezing point depressions and osmotic pressures of the sap of herbs on Ephraim Canyon watershed, central Utah, classified by species, type, situation, altitude, and date of collection—Continued

[Figures in italics represent average of two or more determinations]

Species and type	Situation	Altitude	Date of collection	Depression of freezing point	Osmotic pressure
<i>Rumex salicifolius</i> Weinm.				Degrees C.	Atmospheres
Spruce-fir.....	Southern aspect.....	Feet 10,000	1921 July 12	0.81	9.8
<i>Saxifraga arguta</i> Don.					
Spruce-fir.....	Northern aspect.....	10,000	July 18	0.86	10.4
<i>Senecio columbianus</i> Greene					
Spruce-fir.....	Northern aspect.....	10,000	July 15	0.96	11.6
<i>Sophia incisa</i> (Engelm.) Greene					
Spruce-fir.....	Southern aspect.....	10,000	July 12	1.07	12.9
<i>Stipa lettermannii</i> Vasey					
Oak brush.....	Sage brush flat.....	7,400	July 26	1.17	14.1
<i>Stipa minor</i> (Vasey) Scribn.					
Spruce-fir.....	Northern aspect.....	10,000	July 21	1.43	17.2
Do.....	Southern aspect.....	10,000	July 14	1.66	20.0
<i>Thalictrum fendleri</i> Engelm.					
Spruce-fir.....	Southern aspect.....	10,000	July 12	1.49	17.9
<i>Trisetum spicatum</i> (L.) Richter					
Spruce-fir.....	Southern aspect.....	10,000	July 15	1.33	16.0
<i>Vagnera amplexicaulis</i> (Nutt.) Morong.					
Aspen-fir.....	Northern aspect.....	8,700	July 24	1.17	14.1
<i>Vagnera stellata</i> (L.) Morong.					
Spruce-fir.....	Southern aspect.....	10,000	July 12	1.17	14.1
<i>Valeriana ceratophylla</i> (Hook.) Piper					
Spruce-fir.....	Southern aspect.....	10,000	July 12	0.96	11.6
<i>Valeriana occidentalis</i> Heller					
Spruce-fir.....	Northern aspect.....	10,000	July 18	0.89	10.7
<i>Viola linguaefolia</i> Nutt.					
Spruce-fir.....	Southern aspect.....	10,000	July 12	1.09	13.1
<i>Viola neomexicana</i> Greene					
Spruce-fir.....	Northern aspect.....	10,000	July 18	0.82	9.0
<i>Wyethia amplexicaulis</i> Nutt.					
Aspen-fir.....	Western aspect.....	8,600	July 26	1.83	22.0
<i>Zygadenus venenosus</i> S. Wats.					
Spruce-fir.....	Northern aspect.....	10,000	July 21	1.10	13.2

## CORRELATION AND DISCUSSION OF RESULTS

## INTERDEPENDENCE OF SAP DENSITY AND WATER RELATIONS

Of the fundamental relationships indicated by the sap concentrations of the individual species in Tables VII to IX and the summaries in Table X the most significant are connected with the water relations within the plant and between the plant and its environment. Some are capable of direct measurement and may be readily correlated, while others can not as yet be measured with precision and are accordingly difficult to interpret.

A study of the easily measured environmental factors—especially precipitation, the changes in the available moisture and the evaporation on those sites which were under investigation in the aspen-fir type in Big Cottonwood Canyon—indicates that the ability of the plant to maintain a proper balance between absorption and transpiration, even at the intermediate elevations of the aspen-fir type, is of vital importance; increasingly so with lower elevations. The Ephraim Canyon series of tests, the results of which are shown in Tables XI, XII, and XIII confirm these relations.

The Wasatch Mountain series of determinations are segregated by growth forms and species in Tables VII, VIII, and IX, while those for the Ephraim Canyon series are given in Tables XI, XII, and XIII.<sup>14</sup> In the case of duplicate tests made at the same time merely the averages of the determinations are given. The type, situation, altitude, date of collection, depression of the freezing point in degrees C. ( $\Delta$ ), and the osmotic pressure in atmospheres (P), are given for each determination. Inasmuch as the thermometric readings were taken to thousandths of a degree the depressions were also computed with the same accuracy, but the osmotic pressures were interpolated from the Harris and Gortner tables (46, 47); the freezing point depressions were rounded off to the nearest hundredth and the osmotic pressures to the nearest tenth, greater precision being inconsistent with the experimental error, incidental to the collection of the samples. The osmotic concentrations for all Wasatch Mountain species upon which determinations were made are summarized in Table X by type, growth form and season of the year.

From the investigations of Livingston and his associates (84, 85, 88, 89) it is evident that when the soil is unable to supply moisture as rapidly as the root system of the plant can absorb it, soil moisture then becomes a limiting factor in plant growth. From the standpoint of moisture supply, a soil is satisfactory for the growth of a plant when it can supply water to the absorbing surfaces of the root system fast enough to meet the vital activities of the plant. Osmosis, which plays such an important role in the ascent of sap, plays an equally important part in the absorption of soil moisture and solutes. A very important relation is therefore set up between the density of the soil solution and the density of the sap in the roots. The osmotic concentration of the cell sap of the roots is closely correlated with the concentration of the soil solution which in turn is dependent upon the amount of moisture in the soil. Shull (116) has, in fact, found that roots have an osmotic concentration somewhat above that of the soil solution of a soil in which the water has been reduced to the wilting coefficient.

This suggested that for the present investigation a comparative determination of the cell sap density of leaves and roots would afford a valuable index of soil moisture content, since the frequently important variables of soil fertility

<sup>14</sup> The species about which there was some doubt as to their specific identity were referred to the National Herbarium for confirmation by specialists.

would be automatically eliminated.<sup>15</sup> Two comparisons between the density of the sap of second-year leaves of 2-2 transplants<sup>16</sup> of western yellow pine and that of the smaller roots of the same plants were accordingly made, one in July and the other in September. One lot of the roots was thoroughly washed in distilled water and air-dried to practically the same condition as that originally obtaining on their surface. From another lot as many as possible of the soil particles were carefully removed without washing the roots. Both lots were packed into test tubes and subjected to the same technique of freezing and pressing as the leaves. The results of the tests are given in Table XIV.

TABLE XIV.—*Comparison of sap densities in leaves and roots*

	Depression of freezing point	Osmotic pressure
	Degrees C.	Atmos- pheres
July 27, 1920:		
Leaves.....	1.35	16.2
Roots (washed in distilled water).....	1.17	14.1
Roots (unwashed).....	1.03	12.4
September 20, 1920:		
Leaves.....	1.48	17.8
Roots (washed in distilled water).....	1.36	16.4

These data show that the density of the sap is lower in the roots than it is in the tops of the same plants.<sup>17</sup>

It is of interest to note that the density of the cell sap changes during the day, increasing as the day advances and decreasing toward evening. The daily minimum occurs in the early morning, indicating an apparent relation between sap concentration on the one hand and photosynthesis on the other. This suggests the observation of Bose (7) who has found that the effects of light and warmth are antagonistic. The former induces a retardation and the latter an acceleration of growth. In fact, in the later or higher stages of plant succession, where competition for light is intense, the plant which is photosynthetically the most efficient is most likely to survive because of its greater food supply.

This is corroborated by the results obtained in the present study. The leaf sap of three-year-old seedlings of Douglas fir growing in the Cottonwood Nursery under half shade in July gave a depression of the freezing point of 1.39° C., denoting an osmotic pressure of 16.7 atmospheres as compared with a freezing point depression of 1.59° C. and a corresponding osmotic pressure of 19.1 atmospheres in adjacent unshaded seedlings of the same age and species. In September tests on sap of the same two lots of seedlings gave very similar results. The shaded seedlings showed a freezing point depression of 1.34° C. and an osmotic pressure of 16.1 atmospheres, while the sap of the unshaded seedlings depressed the freezing point 1.50° C., indicating an osmotic pressure of 18.0 atmospheres. The herbaceous and even some of the ligneous plants growing under a dense forest canopy were also found to possess relatively low concentrations.

There is, moreover, a direct relation between growth form and sap concentration: The leaves of ligneous plants were found to have a higher osmotic concen-

<sup>15</sup> The recent work of Bouyoucos and his colleagues (8-9, 10-13), Hibbard and Harrington (66), and Keen (70) is of considerable interest in this connection.

<sup>16</sup> Nursery stock grown two years in the seed bed and two years in the transplant bed.

<sup>17</sup> This conclusion is confirmed by the results of investigations by Hannig (44), Hibbard and Harrington (65), and McCool and Millar (90, 91). The latter investigators have also thrown additional light on the relation between the concentration of the cell sap and environmental conditions.

tration than those of herbaceous species.<sup>18</sup> Variations in growth form are in turn dependent upon environmental conditions. Some species which are ordinarily trees may develop as shrubs in certain environments as, for example, the dwarfing of tree growth at timber-line in parts of the Rocky Mountains.

The existence of an osmotic gradient within the plant, which has been referred to above, is now readily demonstrable in the leaves of tall trees.<sup>19</sup> Determinations made in the present study showed higher concentrations in the top of the tree. The osmotic concentration of the cell sap of leaves increases from lower to higher levels in the same plant. A further study of this phase was made on the Wasatch National Forest, where the two-year-old leaves of 10 trees of western yellow pine growing in Whipple Gulch yielded the following data:

Height of leaves selected	Depression of freezing point in degrees C.	Osmotic pressure in atmospheres.
Base of trees.....	1.44	17.3
Top of trees (average height above ground, 40 feet).....	1.54	18.5

These data afford additional proof of the soundness of the above-stated proposition. It is a general principle that conditions are more favorable to the formation of soluble carbohydrates in the upper parts of the crowns of trees. It is therefore reasonable to assume that the greater osmotic pressures are regularly developed in the tops and that smaller pressures exist at the bases of the crowns, where these substances are not commonly stored. Variations in the sugar content of the leaves due to differences in illumination would also be expected to cause material fluctuations in the osmotic concentration of the sap. These sap-density figures consistently confirm these postulates. The fact that the higher osmotic pressures occur in the tree tops is in accord with Dixon's (23-24, 32-33) theory that the transpiration stream is set in motion through the plant by secretory action occurring in the leaf cells or by evaporation and capillarity at their surfaces drawing water from the tracheae. The condition of saturation surrounding these cells determines which agency shall be effective. With normal transpiration unaffected by root pressure, the cohesion of the sap is sufficient to explain the transmission within tracheae of the tension downward, and the resultant ascent of the sap. With the evaporation of water through the stomata a somewhat higher concentration of the sap is produced in the cells immediately adjacent to the stomata. This upsets the osmotic equilibrium and a current of sap is set in motion, extending all the way from the soil to the stomatal cells of the topmost leaf of the tree. Dixon (32) has shown that the tensile strength of the sap is much in excess of the tension necessary to raise the sap to the top of the tallest trees. Further, the osmotic pressures which he has measured have always been found adequate to resist the tension caused by the transpiration stream. In many cases, however, other factors enter, such as the presence of an unusually large amount of stored soluble carbohydrates or a high concentration of the soil solution, which develop pressures greatly in excess of those required by the transpiration stream alone.

<sup>18</sup> The same thing has been observed by several other investigators (30, 40, 50, 54, 56, 59, 60.)

<sup>19</sup> On the basis of determinations of the osmotic concentration of the leaf sap at different levels above the ground, made by the plasmolytic method, Ewart (38) advanced the theory that the density of the leaf sap increases from lower to higher levels in the same plant. Further observations have been made by Dixon and Atkins (25, 36), and Harris, Gortner and Lawrence (53), who used the refined freezing-point depression method.



One of the strongest arguments in support of the theory that water intake by the plant depends on the osmotic concentration of its tissue fluids is to be found in a comparison of the saps of parasite and host. The osmotic concentration determinations on parasitic Loranthaceae in relation to those of their hosts, discussed on pages 897-898, show that osmotic pressure is one of the most important forces by which the solutes obtained by the parasite are withdrawn from the tissues of the host.

The facts just presented are regarded as fully justifying the assertion made on an earlier page that an intimate relation exists between the concentration of the cell sap and the water relations of the plant. The correlation of data on water relations is facilitated by comparative studies of sap density. Sap density may be used as a convenient measure for expressing with an accuracy sufficient for practical purposes the moisture conditions of different sites and different forest types. Such an index must not be confused with the underlying causal factors which produce it. The osmotic concentrations characteristic of species adapted to different sites serve as criteria of these sites, and, in view of the fact that all of the activities of the protoplasm must take place in the environment of the cell sap, the relative densities of the sap of a species growing under diverse conditions evidently indicate the manner in which those conditions affect the species. In short, the concentration of the cell sap is an expression of the biological requirements of a species since it indicates the ability of a plant to withdraw water from the soil.

### SEASONAL VARIATION IN SAP CONCENTRATION

Table VII shows that the sap concentration for a given species varies not only from site to site but on the same site from season to season. The September concentrations were in general considerably lower than those found in July, a fact which is directly related to the influence of the environment upon the plant. Dixon and Atkins (35) have pointed out that since the major part of the osmotic pressure in the cell sap of plants is due to dissolved carbohydrates, it is evident that the variations in the sap density are largely due to fluctuations in the synthesized solute content of the cells. These differences, therefore, are dependent not only upon the moisture content of the soil but also upon atmospheric conditions which may materially influence current sap concentrations through their effects upon the photosynthetic activities of the plant and the production of carbohydrates. In the course of a year the plant passes through a definite cycle of changes directly dependent upon these varying conditions.

A number of factors combine to start growth in the early spring. The soil is abundantly supplied with water; the snow is melting under the influence of rising temperatures and spring rains; the intensity and duration of the sunshine are increasing; and yet transpiration is still moderate. All of these conditions favor the maintenance of a low sap density. With the advance of the season and the advent of midsummer the supply of available soil moisture is usually much depleted, as has been shown, and the rate of water loss from the plant through transpiration has been materially increased by the occurrence of the highest temperatures of the season and the greatest intensities of sunlight. Summer is, furthermore, the season of minimum rainfall. All these factors unite to produce the maximum sap densities of the growing season. It is during this period that death from drought most frequently occurs. To survive, a species must be able to adapt itself to the changing conditions and to resist the adverse stresses. It can do this by a reduction of transpiration or by such an increase of sap density that absorption continues even in spite of an increasingly concentrated soil

solution.<sup>20</sup> Species unable to thus adapt themselves will disappear from the type. As autumn approaches with its lower temperatures and reduced transpiration the fall rains augment the moisture supply. With the return of these favorable conditions the density of the cell sap is again materially lowered. The September determinations were made after the occurrence of several fall rains, of which the results are apparent in the prevailingly low densities compared with those of July.

While winter is the dormant season for growth and, in the main, for photosynthesis also, transpiration continues except during such times as the leaves are actually frozen. In winter the water losses of coniferous evergreen trees with their leaves intact have been shown by Weaver and Mogensen (120) to be relatively no greater than the losses from deciduous trees after leaf-fall. This indicates great water-retaining capacity in the leaves of conifers, many of which are very hardy. With some species, at least, a small amount of photosynthesis doubtless occurs on some of the warmest days, but it is necessarily of short duration.

TABLE XV.—*Sap densities in first and second year leaves of conifers*

Species	Depression of freezing point in degrees C.		Osmotic pressure in atmospheres	
	First-year leaves	Second-year leaves	First-year leaves	Second-year leaves
<i>Pinus ponderosa</i> .....	1.03	1.11	12.4	13.4
<i>Pinus ponderosa scopulorum</i> .....	1.35	1.38	16.2	16.6
<i>Pinus flexilis</i> .....	0.91	1.29	11.0	15.5
Do.....	0.62	0.79	7.5	9.5
Do.....	0.51	0.54	6.1	6.5
Do.....	0.64	0.67	7.7	8.1
<i>Pinus sylvestris</i> .....	1.17	1.25	14.1	15.0
Do.....	0.66	0.75	8.0	9.0
<i>Pinus austriaca</i> .....	0.81	1.19	9.8	14.3
<i>Pseudotsuga taxifolia</i> .....	0.64	0.66	7.7	8.0
Do.....	0.76	0.92	9.2	11.1
<i>Abies concolor</i> .....	0.70	0.83	8.4	10.0
Do.....	1.02	1.35	12.3	16.2
Do.....	1.18	1.28	14.2	15.4
Do.....	1.39	1.47	16.7	17.7
Do.....	0.96	1.04	11.6	12.5
Do.....	0.57	1.06	6.9	12.8
Do.....	0.54	0.64	6.5	7.7
<i>Abies lasiocarpa</i> .....	0.75	0.86	9.0	10.4
Do.....	1.20	1.35	14.4	16.2
Do.....	1.21	1.30	14.6	15.6
Do.....	0.8	0.85	9.8	10.2
Do.....	0.85	1.12	10.2	13.5
Do.....	0.60	0.74	7.2	8.9
<i>Picea parryana</i> .....	0.80	1.02	9.6	12.3
Do.....	1.21	1.34	14.6	16.1
Do.....	1.09	1.40	13.1	16.8
Do.....	1.05	1.16	12.6	14.0
<i>Picea engelmanni</i> .....	1.17	1.29	14.1	15.5
Do.....	1.19	1.40	14.3	16.8
Do.....	0.86	0.95	10.4	11.4
<i>Picea excelsa</i> .....	0.86	1.00	10.4	12.0

The December and January collections were made while the leaves of the conifers and evergreen shrubs were frozen solid; the temperatures varying from 5° to 15° C. below the freezing point. These winter determinations were found to be at variance with the summer and autumn series in that the conifers showed low concentration, while the closely associated evergreen shrubs exhibited high concentration. Furthermore, the sap densities of the shrubs increased with increase in elevation, a complete reversal of the results secured in the series

<sup>20</sup> The increasing physical grip of the soil particles on the soil solution may also be important.

of the preceding July and September. Inasmuch as these results were so completely at variance with the other two series a special study was made to determine the underlying causes. The results of this investigation are incorporated in the subsequent discussion of "Seasonal Changes in Food Reserves."<sup>21</sup>

It has been shown that young, newly developed leaves have uniformly lower sap densities in July than one-year-old leaves. In all cases the first and second year leaves were taken from the same twigs. In September the relative densities were found to be variable; about one-half of the species showed higher densities in the leaves of the current year and half in those of the second year. By December and January, however, all of the species tested showed uniformly higher densities in their second-year leaves, as shown in Table XV.

These data are in general agreement with the preceding discussion and also with that which is presented in the next section. This difference in density of the sap of the first and second year leaves is possibly due to the greater amount of dissolved carbohydrates in the second-year leaves with resultant higher sap densities.

Another interesting relation was noted in September between yellow and red leaves and normal green ones on the same branches of aspen, large-tooth maple, and chokecherry. The results obtained in Big Cottonwood Canyon are given in Table XVI.

The leaves on these deciduous broad-leaved species were just beginning to take on their gorgeous autumnal colorations. It is probable that in the case of the colored leaves the abscission layer was forming at the base of the petiole and that the greater part of the carbohydrates had already passed from the leaves to the storage organs. This offers a possible explanation for the lower densities in the sap of the colored leaves taken from the same branches as the green leaves.

TABLE XVI.—Sap density as influenced by autumnal color change

Species	Depression of freezing point	Osmotic pressure
	Degrees C.	Atmospheres
<i>Populus tremuloides</i> :		
Yellow leaves.....	1.39	16.7
Green leaves.....	1.83	22.0
<i>Acer grandidentatum</i> :		
Red leaves.....	1.44	17.3
Green leaves.....	1.52	18.3
<i>Prunus melanocarpa</i> :		
Red and yellow leaves.....	1.28	15.4
Green leaves.....	1.75	21.0

It is obvious that the concentration of the sap of any species is subject to wide variations. This is, nevertheless, of great significance in showing that the striking variations in the cell sap concentration of a given species encountered in different seasons and on different sites serve to emphasize the necessity of having a large number of determinations available for the same species on different sites and at different seasons of the year before attempting a correlation of the osmotic concentration of the cell sap with plant activities, dynamically exemplified in growth, distribution, and succession.

<sup>21</sup> It is of interest to note in passing that Lewis and Tuttle (80) found that the maximum sap concentrations are reached in the leaves of *Picea canadensis*, *Pyrola rotundifolia*, and *Linnaea borealis* in the winter, and that the variations are due chiefly to the nonelectrolytes of the sap, the sugar content variation of which followed closely the fluctuations in the sap concentration.

## SEASONAL CHANGES IN FOOD RESERVES

The low osmotic concentrations of the conifers and the high concentrations found in the evergreen shrubs in the middle of the winter show a strong tendency toward a complete reversal of the results of the summer and autumn determinations. This was especially noticeable in the Taylor Canyon series. Douglas fir, white fir, alpine fir, and limber pine showed unusually low densities, while the two junipers were abnormally high. The evergreen shrubs, *Artemisia tridentata*, *Cercocarpus ledifolius*, *Odostemon repens*, *Pachystima myrsinites* and *Ceanothus velutinus*, showed strikingly high winter sap densities which increased with an increase in elevation. These results were confusing until micro-chemical tests for starch and oil were made on five conifers, Douglas fir, alpine fir, white fir, limber pine, and Rocky Mountain juniper, and the above five evergreen shrubs. The conifers gave a strong oil reaction indicating the presence of large amounts of oil and fatty substances in the leaves, while the evergreen shrubs gave only faint oil reactions with the common osmic-acid tests.<sup>22</sup> No substantial increase in the oil content of the evergreen shrubs was observed during the winter.

Starch was not detected in any of the conifers or evergreen shrubs, in either January or February, when treated with an aqueous solution of potassium iodide and iodine (98, p. 28). With the rise of the sap in the spring strong starch reactions were obtained even without preliminary swelling of the starch grains with potassium hydroxid. These tests led to the conclusion that with the advent of cold weather in the autumn and early winter a large part of the starch in the conifers is converted into oil or fatty substances which are osmotically inactive and form emulsions having low osmotic concentrations. In the evergreen shrubs which showed little or no oil present the starch was evidently converted into soluble sugars, thereby materially increasing the osmotic concentration of the cell sap.<sup>23</sup> In the late winter or early spring a reconversion of starch and an apparent decrease in the amount of oil occurs throughout the tissues of the stem. This converted starch is apparently consumed in the formation of the spring growth, and it is not until summer that a fresh supply begins to be deposited.

Starch, when found during the winter, was most abundant in regions remote from centers of conduction and in cells with thin or unligified walls or large

<sup>22</sup> The definite increases noted in these observations on the conifers accord with those of Fischer (39), Preston and Phillips (103), and Sinnott (117), who have listed the majority of the conifers of eastern North America among their "fat trees."

<sup>23</sup> These conclusions are in general agreement with the findings of Fisher (39), LeClerc du Sablon (78), Niklewski (96), Preston and Phillips (103), Sinnott (117), Mer (92), Mitra (94), Miyake (95), Tuttle (118), (119), Coville (22), Petersen (100) and others who have discovered many important facts in regard to the character and seasonal changes of the food reserves in woody plants. Fisher (39), Niklewski (96), and LeClerc du Sablon (78) found that the amount of sugar varies during the year. Lidforss (81-82) found that with most of the wintergreen plants of South Sweden, during cold weather at least, the starch is almost entirely changed to sugar, although on the return of warm weather starch may be again deposited in the cells. Mitra (94) determined that the maximum starch content in apple stems and roots is reached in October and November and that the total sugars increased in January and March. Rigg (107) also has observed the accumulation of sugar at the expense of starch, during the winter, in the evergreen angiosperms of the Puget Sound Region. It is important to note that Coville (22) found that the exposure of plants to cold results in the transformation of stored starch to sugar, with the consequent development of high osmotic pressures. He advances the theory that, during the process of chilling, the starch grains stored in the cells of the plant are at first separated by the living active cell membranes from the enzyme that would convert the starch into sugar, but that when the plant is chilled the vital activity of the cell membrane is so weakened that the enzyme permeates it, comes in contact with the starch and transforms it into sugar. Sinnott (117) suggests that the ease with which water or substances soluble in water have access to the plant cell is probably a determining factor in the extent to which starch is changed to sugar, and that differences in the type of food reserve may be due to differences in water content of the various storage cells, resulting in enzyme activity, or differences in the ease with which enzymes have effective access to the storage cells.

pits. However, it is evident that non-nitrogenous materials are most commonly stored in the form of starch and frequently this is partially or wholly replaced during the winter by sugar or fats. A sudden increase in temperature during the winter or early spring causes a reformation of starch. Under favorable conditions this may occur more than once in the course of a single winter.<sup>24</sup> There is furthermore, a distinct advantage in the economy of the plant in producing, from the same nutrient material, substances of higher or lower osmotic concentration according to the character which metabolism assumes.<sup>25</sup>

The supply of reserve food is an important factor in the inception of early spring growth. The reserve food materials stored up in the autumn are probably largely utilized in leaf and also in blossom formation, when the latter precedes leaf formation. Growth does not begin simultaneously in the evergreen and deciduous trees of the Intermountain Region. It would appear reasonable to assume that diameter growth proper, as distinguished from any preliminary swelling of the tissues, may be delayed in the deciduous trees until the new leaves have developed and have become sufficiently active photosynthetically to supply the requirements of rapid cell formation. Evergreen conifers, on the other hand, have an adequate amount of living leaf tissue to supply the requisite materials for growth as soon as growing temperatures are reached in the spring. But, even in conifers, the stored food materials are principally, if not wholly, consumed in the incipient stages of the current growth while growth during the main part of the growing season is largely influenced by other environmental factors, notably precipitation, since this is the chief determinant of the moisture supply in this region (75, 76).

Since winter sap densities are materially altered by transformations in the food reserves, any determinations which are made during the winter must be interpreted accordingly. For example, higher winter sap concentrations should not be interpreted as necessarily indicating greater drought resistance in one species than in another. Because of the material changes in the food reserves following the advent of cold weather it would appear inadvisable to use winter determinations alone in a correlation of sap density with environmental conditions. In the Intermountain Region, at least, the dry part of the growing season is the most critical from the standpoint of plant distribution and succession. Sap densities common to this period are therefore of greater significance than those of winter for correlation with the phenomena, which are largely dependent upon environmental conditions.

## SAP DENSITY IN RELATION TO FOREST DISTRIBUTION AND PLANT SUCCESSION

The sap density of any single species shows variations corresponding to the degree of xerophytism of the forest types or associations in which that species is growing, especially when the determinations are made at or near the critically dry part of the growing season. Although a plant with low sap concentration can procure the necessary water from soil having a high moisture content, the total amount of available soil moisture is not always in itself the limiting factor in the struggle of a seedling to become established. Its success often depends principally on its ability to exert, through the concentration of the cell sap, an osmotic pressure sufficient to absorb water from the soil and at the same

<sup>24</sup> These observations have been confirmed by Mer (92).

<sup>25</sup> Pfeffer (102, p. 134-147) has pointed out that reserve food materials may be stored up in an insoluble form as starch, oil or proteid without causing any increase in the osmotic concentration of the cell sap, but that even the concentration of two molecules of a monosaccharide to one of a disaccharide may result in reducing the osmotic concentration by one-half.

time maintain an economically efficient transpiration rate under temperature conditions favorable to photosynthesis. This dual relation between absorption and transpiration, then, is more often the determining factor in the composition of the stand than absorption alone. It is to this that the several forest types are due.

Evidently, therefore, (a) the water relations of the plant have a vital bearing on the succession of plant associations and (b) the causes of these phenomena are associated with the osmotic properties of the plant juices and the soil solution. The densities of the sap of all species tested in the main Wasatch Mountains, which are averaged by types, growth forms and seasons in Table X, illustrate this point. The average densities of the sap of all species tested in each type in the Ephraim Canyon series in July, 1921, are given in Table XVII.

TABLE XVII.—Average sap densities by types for the Ephraim Canyon series

Type	Depression of freezing point	Osmotic pressure
	<i>Degrees C.</i>	<i>Atmospheres</i>
Greasewood-shadscale.....	4.29	51.4
Sagebrush.....	2.35	28.2
Pinon-juniper.....	1.78	21.4
Oak brush:		
South aspect.....	1.66	20.0
North aspect.....	1.36	16.4
Average.....	1.47	17.7
Aspen-fir.....	1.25	15.0
Spruce-fir:		
South aspect.....	1.25	15.0
North aspect.....	1.05	12.6
Average.....	1.16	14.0

These averages, together with the principal climatic factors, are plotted in figure 5, which further emphasizes the correlation between site and sap density. The sap of sagebrush, the extreme range of which covers several types, shows the same characteristic decrease in density with an increase in altitude. (Fig. 5.) A comparison of the opposite north and south aspects at the same elevations in the oak brush and spruce-fir types also shows a decrease in sap density corresponding with an improvement in site conditions. The plants have the lower densities on the northern aspects, which in these two cases are the more favorable for plant growth. Similar comparisons can also be drawn from the majority of the species listed. The common progressive succession is from the high osmotic pressures in the drought-resistant species of the pinon-juniper and chaparral zones of the lower mountains, through the western yellow pine, aspen-fir, and logdepole pine types composed of species having successively lower sap concentrations, to the climax spruce-fir type of uniformly low osmotic pressures. The establishment of a forest cover is conducive to greater stability and the development of progressively lower osmotic concentrations in the perennial species. It is somewhat different in the case of annual herbs which are capable of completing their life cycle in shorter periods, often during a rainy season when conditions are optimum for vegetative growth.

A correlation of plant succession with the underlying casual factors shows that the succession is from drought-resistant species toward species of less drought resistance as the atmospheric and soil moisture increases and produces greater stability and lower sap densities. It is evident that the existence of a high osmotic concentration of the soil solution tending toward physiological dryness of the soil would prove most detrimental to species of low sap concentration by cutting off the moisture supply. Shallow-rooted species having

low sap concentrations would be the first to be adversely affected by periodic droughts. The depth of penetration, lateral spread, general character of the root system and volume of soil penetrated by the roots, as well as the capacity of the species to absorb moisture, are all important in determining the adaptability of the species to a given site. These factors also influence the amount of moisture available to the plant.

The cutting off of the supply of available soil moisture through freezing of the soil is occasionally a limiting factor. Although the Wasatch Mountains are usually covered during the winter by a mantle of snow often several feet deep, under which the soil is seldom frozen, windswept ridges and southwestern aspects are intermittently bare of snow for sufficient intervals to permit freezing to considerable depths. Deep freezing of the soil, especially when accompanied by high winter evaporation stresses, is the chief factor accountable for the limited number of native species found on these sites. The vegetative cover is generally composed of a few species having deep taproots which extend below the depth to which the soil freezes. The freezing of the soil accompanied by high evaporation and consequent excessive transpiration tends to exclude coniferous species and others having low sap concentrations. Adverse factors would naturally be more markedly inimical to the establishment and growth of the young seedlings than to the growth of those plants which have already become established and it is the conditions influencing reproduction which ultimately determine the composition of a given forest type or association.

The zonation of plant associations and their alternation on opposing north and south aspects can be logically explained on the basis of the effect of environmental conditions on the known physical properties of the cell sap of their component species. Although other factors, such as shade or other means of protection, may cause more or less sporadic occurrences, it will usually be found that they do so through mitigating the environmental conditions during the seedling and sapling stages.

A discussion of sap density in relation to forest distribution and plant succession would not be complete without considering its practical application. Foresters have long recognized that the denudation of an area bearing a certain type of vegetation may be followed by an entirely different type of cover; for example, aspen often temporarily replaces Douglas fir and Engelmann spruce after their removal by burning. Douglas fir is frequently followed by lodgepole pine on burns. To a less extent silvicultural management cuttings are followed by a natural succession, as shown by Bates (4) and Weidman (121). When a mixed stand of Douglas fir, western larch (*Larix occidentalis*), grand fir (*Abies grandis*), and Engelmann spruce is cut over under certain conditions, the first reproduction which appears is sometimes almost exclusively the inferior grand fir, the more valuable species coming in again only after a considerable lapse of time. In order to control the succession in such cases, the forester must use to advantage the known laws governing plant succession, adapting his silvicultural practice to them. The main available means of doing this is by adapting the degree or method of cutting to the particular case. In some stands, for example, it might be economically desirable to retain the present condition, which may not be the ultimate climax stage; in others the natural succession might be hastened; in still others it may be desirable, if possible, to retard it. The maintenance of a particular composition or density will depend upon a knowledge of the competition and reaction of the dominant species and the individual trees in the stand, and the relation of these to the successional development. In determining what species will permanently occupy any given site the conditions must be known which will induce the minimum moisture supply and the maximum osmotic concentration likely to be developed in any climatic cycle.

Sap density also vitally affects the success of artificial reforestation in regions of deficient and poorly distributed rainfall. Only those species can succeed which can maintain a sap concentration high enough to provide an osmotic gradient sufficient to maintain the transpiration stream. In other words, the plant must be able to absorb moisture from the soil to replace the water lost through evaporation from the leaves. It would, therefore, appear unwise to attempt to grow on any site a species having a smaller range in sap concentration than that exhibited by native species normally present on the site; otherwise the introduced species will be unable to succeed in its new environment.

GROWTH AND REPRODUCTION IN RELATION TO SAP DENSITY

Osmotic pressure may exert a decided influence on the growth and reproduction of plants. Livingston (83) has shown that growth is accelerated in weak soil solutions and is retarded in concentrated ones. Reproduction in some cases appears to be almost entirely dependent upon the osmotic pressure of the soil solution surrounding the roots. The density of the sap increases as the season advances due largely to the accumulation of solutes, although there is some evidence that diminished absorption of moisture from the soil is partially responsible for the increase of sap density. Reed (106) has found that the growth and sap concentration in young trees vary in opposite directions; that is, low concentrations of the sap in the shoot appeared to be associated with abundant water intake and rapid vegetative growth, while higher concentrations were associated with slow growth and bud formation.

It was found in the present investigation that both age and the conditions of growth exercise a profound influence on the osmotic pressure of the cell sap. Newly developed leaves of the two varieties of western yellow pine, lodgepole pine, and western white pine showed lower concentrations in July than one-year-old leaves taken from the same plants, all of which were growing in the Cottonwood Nursery under identical exposure. The results are given in Table XVIII.

TABLE XVIII.—Effect of maturity on sap density in pine leaves

Species, and age of leaves	Depression of freezing point	Osmotic pressure
<i>Pinus ponderosa</i> :	Degrees C	Atmospheres
Month-old leaves.....	1.39	16.7
Year-old leaves.....	1.55	18.6
<i>Pinus ponderosa scopulorum</i> :		
Month-old leaves.....	1.46	17.6
Year-old leaves.....	1.57	18.9
<i>Pinus contorta</i> :		
Month-old leaves.....	1.29	15.5
Year-old leaves.....	1.42	17.1
<i>Pinus monticola</i> :		
Month-old leaves.....	1.07	12.9
Year-old leaves.....	1.41	17.0

The young succulent leaves, when subjected to the regular technique in expressing the sap, yielded three to four times the quantity that was secured from the same amount of mature leaves under the same pressure. The sap of the young leaves was also thin and almost colorless, resembling a sugar solution and largely lacking the characteristic resinous odor of coniferous leaves, while the sap from the mature leaves was thicker, possessed the resinous odor, and was of a bright green color due to the presence of some of the chlorophyl matter which was expressed with the sap.

It is evident not only that periods of retarded growth correspond to periods of high sap concentration but also that in trees found typically in regions of little



moisture, as, for example, the juniper and western yellow pine types, a slower rate of growth and abnormally high osmotic pressures are concomitant. A low concentration of the soil solution would in turn favor selective absorption through the action of the osmotic gradient and still permit the maintenance of a relatively low sap concentration in the leaves of the plant desirable from the standpoint of stimulating rapid growth.<sup>26</sup> Forest trees of course make their best growth on the best sites, the sites from the best to the poorest being merely a relative classification of the growth capacity of different habitats as influenced by environmental factors. The lowest sap concentrations for a given species are found on those sites on which the environmental conditions are most favorable for rapid growth.

The intimate relation which exists between sap density and growth rate is emphasized in the present study. The growth rate of Douglas fir and white fir in Big Cottonwood Canyon is more rapid on the south aspect than on the opposite north aspect at an elevation of 7,500 feet where the aspen-fir type meets the chaparral type. The competition of the open stand of trees on the south slope is in this case nowhere intense, either with one another or with the shrubby species. The trees are rooted in a soil layer below that of the bulk of the shrubs; one in which the soil moisture supply is abundant, as evidenced by the presence of aspen, an indicator of an ample supply of subterranean moisture. On the north aspect, on the other hand, growth is retarded by competition for the soil moisture in the lower soil layers, not only between individuals in a denser stand of forest trees, but also between the trees and the dense stand of deeply rooted shrubs. Higher sap concentrations are also encountered on this north aspect. The south aspect is a striking example of a low sap concentration of Douglas fir and white fir associated with a relatively high transpiration rate. This can be fully explained by the presence of an abundant supply of subterranean moisture. The correlation of a low sap density with this rapid growth rate of well established sizeable trees should not be confused with the question of favorableness or unfavorableness of site from the standpoint of the establishment of reproduction. Practically no seedlings of either species are being established on the south aspect on account of the meagerness of the moisture in the upper foot of soil and the excessive evaporation, while on the north aspect with its favorable moisture conditions near the surface there is found an abundance of advance reproduction.

A somewhat similar comparison can be drawn in the case of the spruce-fir type. A study of Table VII shows that the density of the cell sap increases as timber line is approached on ridge tops and south aspects above 10,000 feet in elevation. With this increase in sap density a falling off in rate of growth is also apparent in Engelmann spruce, alpine fir, and limber pine on these sites.

## INFLUENCE OF STRUCTURAL AND PHYSIOLOGICAL FACTORS

The structure of most plants varies with the site and even with the fluctuating environmental factors of the same site. The leaf, because it is the seat of important physiological activities of the plant and because of its modification by external factors, has long been the field for numerous investigations. The majority of such studies have been confined to the morphology and histology of the leaf, independent of its relations to environmental factors. In contrast to these Clements (19), Hanson (45), and Sampson and Allen (112) took into account measured physical factors in relation to leaf structure. The importance of this relationship was repeatedly emphasized in the present study while collecting the

<sup>26</sup> According to Hoagland (66) marked absorption of nutrient materials may take place during all stages of growth when suitable concentrations of the various ions are maintained. Burd (14) has pointed out that for many plants high concentrations of the soil solution at certain stages of growth are probably undesirable.

leaf material for the sap density determinations. Plants on exposed sites are usually thicker, more hairy, have a more highly cutinized and thicker epidermal wall, a more compact palisade parenchyma, and more closely crowded stomata than on less severe sites.

The powers of accommodation or adaptation exhibited by plants are evident in the density of the sap. The extent of adaptation varies with the plasticity of the species. The curl-leaf mahogany (*Cercocarpus ledifolius*), manzanita (*Arctostaphylos pungens*) and mountain myrtle (*Pachystima myrsinites*), which have thick leathery leaves, and *Ceanothus velutinus*, with a very sticky leaf, are species possessing variable sap concentrations. On the other hand, the succulent *Opuntia* and *Sedum* have surprisingly low sap densities. Those plants which possess a low transpiration rate as a result of anatomical modifications, the presence of pubescence or pulverulence, and those plants having pronounced water storage tissue in succulent leaves or stems are capable of maintaining surprisingly low concentrations of the cell sap.

In many plants the major physiological activities, such as photosynthesis, absorption, and transpiration, are materially modified by special protective coverings and by anatomical and even functional adaptations. The rate of transpiration, for example, may be materially reduced by structural modifications. Since osmotic phenomena are so intimately connected with all these vital physiological functions it is clear that an intimate relation also exists between structural adaptations and the density of the cell sap.

Our knowledge of the water relations of some western mistletoes also has been augmented by a study of the osmotic concentration of the sap of these parasites in comparison with that of their hosts. Determinations on the sap of *Razoumofskya douglasii* on Douglas fir in Big Cottonwood Canyon and *R. americana* on lodgepole pine on Beaver Creek, both made in September, 1920, and *R. cryptopoda* on western yellow pine and *Phoradendron juniperium* on one-seed juniper (*Juniperus monosperma*), in August, 1921, at the Fort Valley Forest Experiment Station near Flagstaff, Arizona, are given in Table XIX.

TABLE XIX.—Sap densities of some western mistletoes and their hosts

Species	Depression of freezing point.	Osmotic pressure.
	Degrees C.	Atmos- pheres
<i>Razoumofskya douglasii</i> .....	1.64	19.7
On <i>Pseudotsuga tazifolia</i> .....	0.98	11.8
<i>Razoumofskya americana</i> .....	1.52	18.3
On <i>Pinus contorta</i> .....	1.41	17.0
<i>Razoumofskya cryptopoda</i> .....	1.46	17.6
On <i>Pinus ponderosa scopulorum</i> .....	1.42	17.1
<i>Phoradendron juniperium</i> .....	1.30	15.6
On <i>Juniperus monosperma</i> .....	1.28	15.4

These results indicate that the osmotic density of the sap extracted from the tissues of the parasite is generally greater than that from the mature leaves of the host.<sup>27</sup> A higher osmotic concentration of the sap of the parasite is not a necessary condition for the temporary success of the parasite. The parasite should be able to draw from the relatively dilute solutions in the stem of its host in competition with organs of actually higher osmotic density, except at periods when the supply of available moisture is limited, just as young leaves are able to draw water in competition with old leaves of high osmotic density. The Arizona

<sup>27</sup> The studies of Harris (58) on desert Loranthaceae and those of Harris and Lawrence (49) on the parasitic Loranthaceae of Jamaica accord with the above findings.

tests were made during a period of unusually wet weather, which probably accounts for the small differential between the sap of the parasite and that of the host. Again, osmotic pressure has been found to be one of the fundamental variables among the forces by which solutes are drawn from the tissues of the host, a discovery that has contributed to the knowledge of the physiology of parasitism.

In the preceding pages the great importance of knowing the reactions between the environmental factors and the various physiological functions of the plant have been repeatedly emphasized. The variable results noted in the winter sap density studies could not have been properly interpreted without an examination of the starch content of the leaves. It has been shown that, through the osmotic concentration of the sap, the initial changes preliminary to early spring growth may be detected by the rise and consequent effect on the density of the sap in the leaves of evergreens. The seasonal changes in carbohydrate conversion may also be followed by making use of the osmotic pressure method. Of no small significance is the applicability of the method to the study of the water balance of the plant—changes in its water relations due to moisture supply and water loss.<sup>28</sup>

The fact has been repeatedly emphasized during this investigation that no one factor or group of factors should be singled out, to the exclusion of all others, as an explanation of all physiological phenomena, and furthermore, that the normal physiological activities of the plant can only be interpreted when they are properly correlated with both the climatic and soil conditions of the environment.

#### RELATION OF DENSITY OF SAP TO HARDINESS

The intimate relation of cell sap concentration to drought resistance has already been brought out. There is also an apparent relation to winter hardiness. This has been indicated by a number of investigators who have correlated the transformation of starch into sugar and the resultant increase in the density of the cell sap in the winter with frost resistance. Lidforss (81, 82) and Petersen (100) have shown that a close relation exists between the sugar contents and frost hardiness; the more sugar the less injury from frost. The sugar is believed to protect the living protoplasm against frost injury by lowering the freezing point of the cell contents.<sup>29</sup>

Numerous theories<sup>30</sup> have been advanced concerning the mechanics of frost injury, such as failure of the protoplasm to regain water lost when ice crystals form in the tissues, the precipitation of proteins, or other metabolic changes accompanying low temperature. A number of empirical observations in the Intermountain Region indicate that the injurious effects come when ice crystals form. Wet shoots freeze at a higher temperature than dry shoots. Wet leaves show more serious injury at a given temperature than dry leaves. A period of cold weather immediately following a rain or wet snow is thus more dangerous than a period of cold dry weather. With the approach of spring there occurs an increase in the tenderness of buds.<sup>31</sup>

<sup>28</sup> Bates and Zon (5) have pointed out that the termination of the season's photosynthetic activity may possibly be determined by the same method.

<sup>29</sup> Fischer (39), Le Clerc du Sablon (78), and Niklewski (96) have also found that, while the amount of sugar in the stem varies during the year, the maximum of sugar in winter makes for greater frost hardiness.

<sup>30</sup> A number of these theories are reviewed and a bibliography of 50 citations dealing with the hardening process in plants is given by Harvey (62).

<sup>31</sup> These observations are in accord with the experimental results obtained by Harvey (62, 63), Chandler (17, 18), Rosa (109), Hauch and Ravn (64), Harris and Popenoe (51), and Ohlweiler (97). On the other hand, Salmon and Fleming (111) maintain that turgidity of the tissue as influenced by physiological drought appears to have more influence than sap density on the ability of a plant to resist winter killing.

The young succulent shoots and leaves are much more susceptible to early fall frosts which occur before the process of lignification has been completed. Late spring frosts after the buds have begun to swell are also very injurious and are increasingly harmful as the new leaves and shoots develop. The greater susceptibility of Douglas fir to frost injury than western yellow pine is probably ascribable to the lower temperature at which Douglas fir, as compared with the pine, reconverts the stored sugars into starch in the spring. As pointed out above, this conversion results in lower sap concentrations, which also offers a possible explanation of the relatively greater susceptibility to frost of the true firs than of Engelmann spruce (74).

On the whole, our native trees and shrubs are so intimately adjusted to the environmental conditions to which they have been long subjected that they are well protected from injury by ordinary freezing. On the other hand, many of the species introduced from regions with different environmental conditions are only imperfectly adapted to withstand the local conditions. They frequently grow at times when the native species, through adjustment and adaptation, have become dormant. As a result they suffer severe injury. For example, hardwood species such as green ash, box elder, black locust, and Russian olive have been completely killed by frost at times when the native conifers remained uninjured.

It is probable, therefore, that the amount of sugars present in the leaf, and consequently the density of the cell sap, has a bearing on frost hardness. Furthermore, it is likely that the different species convert starch to sugars at different temperatures. By experimentally determining the relative temperatures at which this conversion occurs in the different species, a further guide should become available for determining their relative frost resistance and their suitability for planting on sites subject to early fall and late spring frosts.

### SUMMARY

The investigation of the density of the cell sap of a large number of plants common to the important forest types of the Wasatch Mountains has yielded results of direct application in forest research. It shows that sap density may be used as an index of site in correlating the great complex of environmental factors with the physiological responses of the plant.

The concentration of the sap of a species is not constant. It may be influenced by any of the environmental conditions affecting transpiration, the products of photosynthesis, or the supply of available soil moisture. Osmotic pressure in plants is more rapidly changed by fluctuations in the moisture conditions of the site than by temperature or light.

Because of the wide range of sites covered in this investigation and the general agreement with the results of studies by other investigators, the following biological principles have been confirmed:

1. Annual herbaceous plants which complete their life cycle before the critically dry part of the growing season, and are therefore not subject to drought conditions, have low sap densities.

2. The concentration of the sap of woody species is much higher than that of herbaceous species.

3. During the growing season the lowest sap densities occur in those forest types which are well supplied with available moisture, whose plants are best adapted to secure it, and in which the complex of conditions is most favorable to plant growth. On the other hand, the highest densities occur on the most adverse (dry or saline) sites.

4. In the winter considerable variation in sap density is encountered, amounting in some cases to complete reversals of the densities of the growing season, due to changes in the soluble contents of the plant cells. In the case of evergreen shrubs, the conversion of starch to sugars materially increases the density of the sap.

5. A thick leaf having a compact structure with a thick epidermis and cuticle tends toward a lower sap concentration through its reduction of water loss from the leaf. The presence of epidermal coverings and hairs on the leaves also makes for lower sap densities.

6. Greater sap densities are generally found in the more drought-resistant species.

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# AN ASCIGEROUS STAGE AND SYNONYMY FOR FUSARIUM MONILIFORME<sup>1</sup>

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## INTRODUCTION

*Fusarium moniliforme* was described by Sheldon (23)<sup>3</sup>, working in Nebraska, in 1904. The fungus was of interest at that time because it was believed to be connected with an ergotism-like disease of domestic animals. State Pathologist Peters (17) and others investigating the disease found no ergot. They discredited other popular beliefs as to its cause. They found that the corn on farms where the disease existed was badly rotted with a pink fungus, while that on farms where the disease had not appeared was not so rotted. Feeding experiments were carried on, and from their results and because of the similar distribution of fungus and disease the conclusion was reached that a causal relationship existed.

Sheldon's contribution in this problem was a painstaking study and a careful description of the fungus itself. It was an unusual *Fusarium* in that it had microconidia in chains. His formal description follows:

Sporodochium sub-effuse, salmon-pink; sporophores, simple or branched, usually opposite; microconidia continuous, oblong-ovoid, moniliform, 6-10  $\mu$  long; macroconidia, falcate, acute, for the most part 3-septate, 25-40  $\mu$  long.

Since 1904 *Fusarium moniliforme* has come up in the literature of this country again and again as a saprophyte or parasite on corn. Holbert and Hoffer (12) mention *Gibberella acervalis* (Moug.) Wr., as one of the factors in root, stalk, and ear rot diseases in Indiana. Norton and Chen (15) report *Oospora verticilloides* Sacc. as a corn seed parasite in Maryland, and associate it with barren distorted stalks and rootrot in the field. The relation of *G. acervalis* and *O. verticilloides* to *F. moniliforme* will be discussed.

Valleau (27, 28) considers *F. moniliforme* an active parasite capable of causing root and stalkrots of corn under laboratory and field conditions, and states that ear and seed infection with the fungus probably is universal. Manns and Adams (13) report the occurrence of *F. moniliforme* as an internal infection of seed corn in seventeen of twenty-one States from which samples for examination had been received, the percentage ranging from 0.4 in North Dakota to 80 per cent in Louisiana. Sherbakoff (25) finds that in Tennessee, as well as in other States, the most common *Fusarium* of corn is *F. moniliforme*. A number of other investigators, as Burrill and Barrett (1), Garman (7, 8), Hewitt (11), and Selby (22), mention a *Fusarium* of corn which may possibly be *F. moniliforme*.

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<sup>2</sup> The writer wishes to acknowledge the benefit of Miss Helen Johann's knowledge of *Fusaria* in general, and experience with *Fusarium moniliforme* in particular and to thank Dr. James G. Dickson for making the photomicrographs and Dr. A. G. Johnson for assistance in revising the manuscript.

<sup>3</sup> Reference is made by number (italic) to "Literature cited," pp. 921-922.

## STUDIES ON CONIDIA

In the present investigations *Fusarium moniliforme* has been found very commonly associated with corn. Extensive isolations have been made from various parts of plants taken from cooperative experimental plots of the United States Department of Agriculture at Bloomington, Ill., and Madison, Wis., at three different times since the fall of 1919, by Helen Johann and the writer. In all, about 350 plants have been studied, and *F. moniliforme* has appeared very frequently.

Some of the cultures of *Fusarium moniliforme* obtained in this way have been kept in the laboratory for observation. The total number isolated was so large

as to make it impracticable to keep all of them. Cultures received from other States, namely, Indiana, Delaware, Maryland, North Carolina, California, Iowa, and Kentucky, have been added to the number. At present the stock includes about twenty-five strains.

It was noticed from time to time that these cultures differed from each other somewhat, and that some of them departed from Sheldon's description along certain lines. The production of sporodochia was one of the most noticeable of these points of difference. Some cultures developed them abundantly, in contrast with others where they were produced rarely or not at all, and in the true sporodochia a large number of 5-septate conidia was found with a corresponding maximum length greater than that given by Sheldon. All of them, however, had the ca-

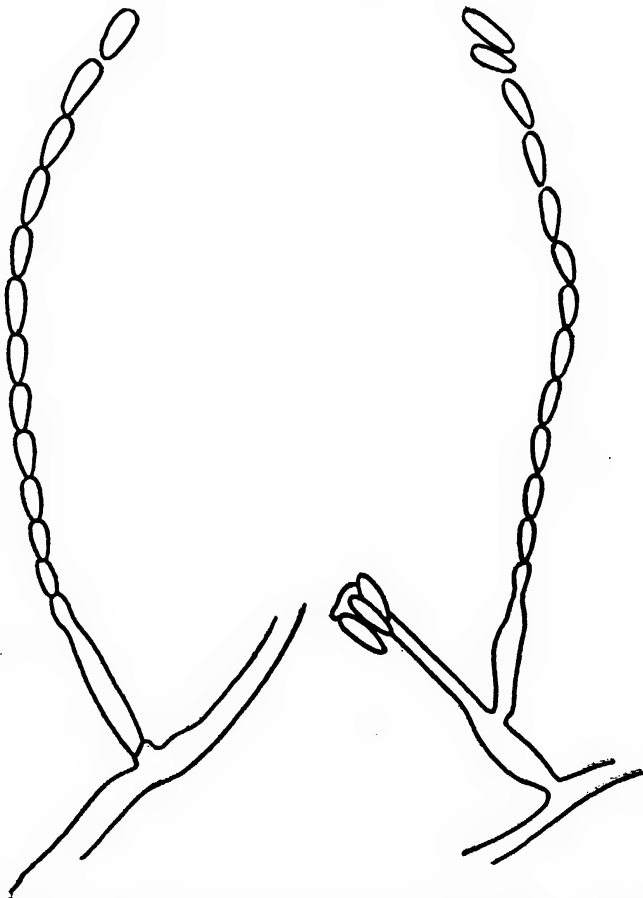


FIG. 1.—Microconidia of *Fusarium moniliforme* Sheldon. Typical of all strains observed. X 1733

tenulate microconidia of *Fusarium moniliforme* (fig. 1) and were confined to the same color range. They showed an early vacuolization and degeneration of macroconidia and mycelium, suggestive of the Section Roseum.

A detailed description of two of these cultures or strains, Y 15 and Y 29, will serve to illustrate some of the variations. Moreover, these two strains are of basic importance in the present discussion.

Descriptions are based on cultures grown at room temperature and on oat agar made according to the formula of Sherbakoff (24, p. 105) unless there is some statement to the contrary. Use of oat agar, potato-dextrose agar, potato-dextrose agar acidified, rye agar, bean agar, steamed rice, potato tuber plugs, Melilotus stems, sterilized wheat heads, sterilized corn stalks, and corn agar for these *Fusaria* has indicated that there is little to be gained from the variety, and that, in general, the oat agar is most satisfactory.

DESCRIPTION OF STRAIN Y 15

The macroconidia of strain Y 15 (fig. 2 and 3) are produced in pseudopionnotes or sub-effuse sporodochia rather than in well-rounded, distinct sporodochia as in Y 29. Ten representative macroconidia from the sources shown in Table I were measured and the averages of these measurements are given in Table I. It will be noted that 3-septate conidia predominate, but in the cases of certain sporodochia considerable percentages of 5-septate conidia were produced.

TABLE I.—Average sizes of 3-, 4-, and 5-septate macroconidia, respectively, of strain Y 15 and percentages of each, from different sources

Age of cultures	Source	Average sizes and percentages of each group in the total number—					
		3-septate		4-septate		5-septate	
Days		Per cent	Microns	Per cent	Microns	Per cent	Microns
69	Aerial mycelium	90	3.15 by 32.18	1	3.00 by 40.00	9	<sup>a</sup> 3.00 by 44.60
42	Sporodochium	54	3.32 by 30.18	0	-----	46	3.00 by 45.27
42	Aerial mycelium	95	3.00 by 30.18	0	-----	5	<sup>a</sup> 2.60 by 41.50
54	-----	79	3.00 by 28.91	10	3.00 by 37.50	11	3.00 by 42.45
36	Pseudopionnotes	100	3.50 by 32.30	0	-----	0	-----
41	Sporodochium	79	3.00 by 34.49	0	-----	21	2.90 by 43.40
55	do	83	3.17 by 35.65	0	-----	17	3.14 by 43.67
	Average	-----	3.16 by 31.98	-----	-----	-----	3.01 by 43.70

<sup>a</sup> Only one seen, not included in average.

Strain Y 15 produces blue-black globoid bodies resembling, macroscopically, the perithecia of *Gibberella saubinetii* (Mont.) Sacc., but smaller, being only 45 to 90μ in diameter. These are especially numerous in potato-dextrose agar, occurring as tightly packed groups or aggregates embedded in the medium. Microtome sections indicate that they are made up of what appears like outer walls of true perithecia, and have a small or no central empty space.

DESCRIPTION OF STRAIN Y 29

The macroconidia of Y 29 (fig. 4) are similar to those of Y 15 (fig. 2 and 3) except that 5-septate conidia are more common in the former strain. They predominate, in fact, and a number of 6-septate conidia also have been noted. As in the case of Y 15, 10 representative macroconidia from the sources noted in Table II were measured and the averages of these measurements are given in Table II. It will be noted that the length of the macroconidia averages somewhat greater in Y 29 even when equiseptate spores are compared.

TABLE II.—Average sizes of 3-, 4-, 5-, and 6-septate macroconidia, respectively, of strain Y 29 and percentages of each, from different sources

Age of cultures	Source	Average sizes and percentages of each group in the total number—							
		3-septate		4-septate		5-septate		6-septate	
Days		Per cent	Microns	Per cent	Microns	Per cent	Microns	Per cent	Microns
61	Sporodochium	-----	<sup>a</sup> 3.50 by 36.60	-----	-----	-----	3.44 by 53.98	-----	-----
69	do	-----	2.92 by 36.34	-----	2.98 by 44.19	-----	2.87 by 53.90	-----	<sup>b</sup> 2.92 by 61.13
42	-----	7	3.00 by 38.03	-----	-----	93	3.50 by 60.83	-----	<sup>c</sup> 3.00 by 69.30
54	-----	5	3.00 by 37.50	12	3.00 by 44.73	81	3.00 by 54.90	2	<sup>d</sup> 3.60 by 63.50
36	Sporodochium	5	3.00 by 37.80	-----	-----	95	3.00 by 58.20	-----	-----
	Average	-----	2.98 by 37.42	-----	2.99 by 44.46	-----	3.16 by 56.36	-----	-----

<sup>a</sup> Only one seen, not included in average.  
<sup>b</sup> Seven measured.

<sup>c</sup> Four measured.  
<sup>d</sup> Two measured.



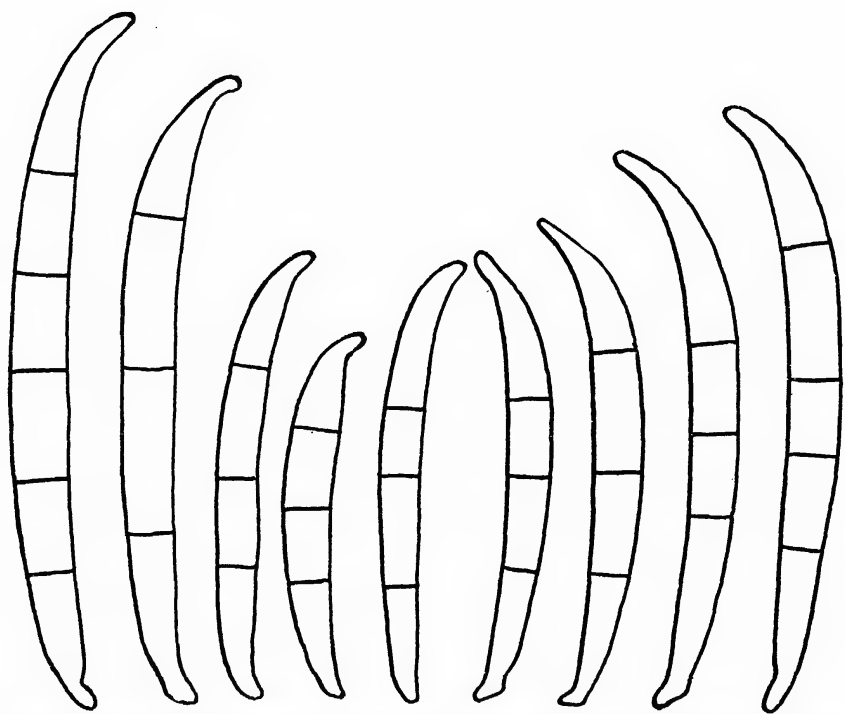


FIG. 2.—Macroconidia of strain Y 15 from pseudopionnotes of a culture 39 days old. X 1733

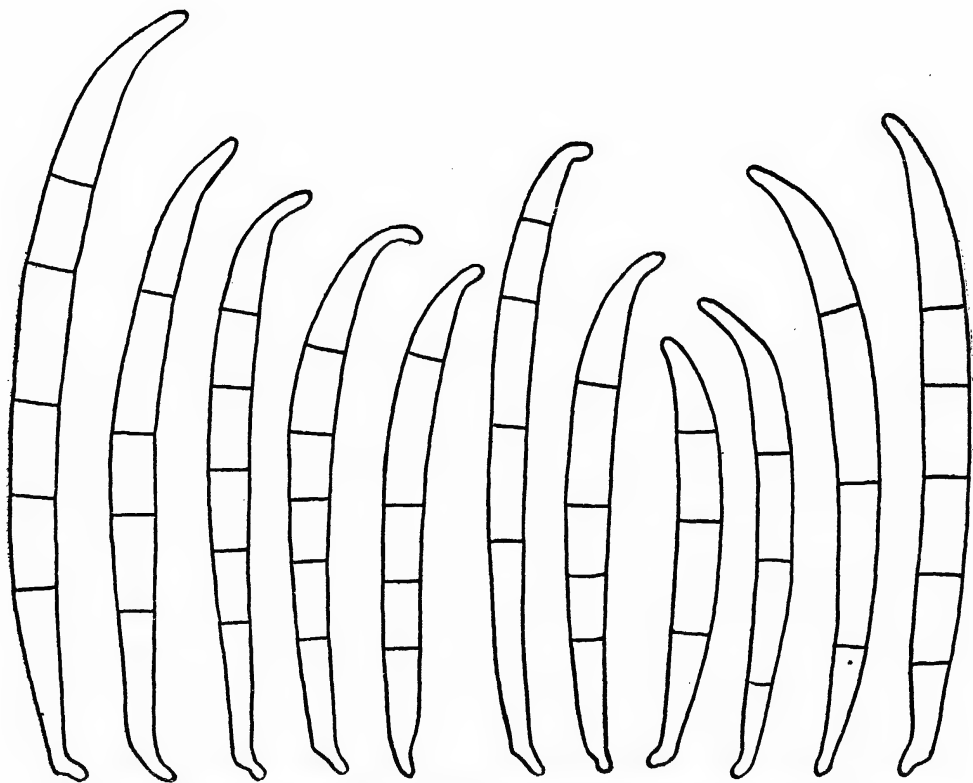


FIG. 3.—Macroconidia of strain Y 15 from sporodochium of a culture 34 days old. X 1733

In addition to these differences in morphology of macroconidia, strain Y 29 differs from Y 15 in that it shows a strong tendency to produce sporodochia of the usual *Fusarium moniliforme* vinaceous-cinnamon (19) and related shades, and also, sometimes produces blue-black plectenchymic sporodochia. Furthermore, strain Y 29 rarely produces the blue-black spherical bodies described for Y 15.

#### PRODUCTION OF AN ASCIGEROUS STAGE IN CULTURE

The blue-black globoid bodies described in Y 15, and found in a few of the total number of strains that have come into the laboratory, suggested the pos-

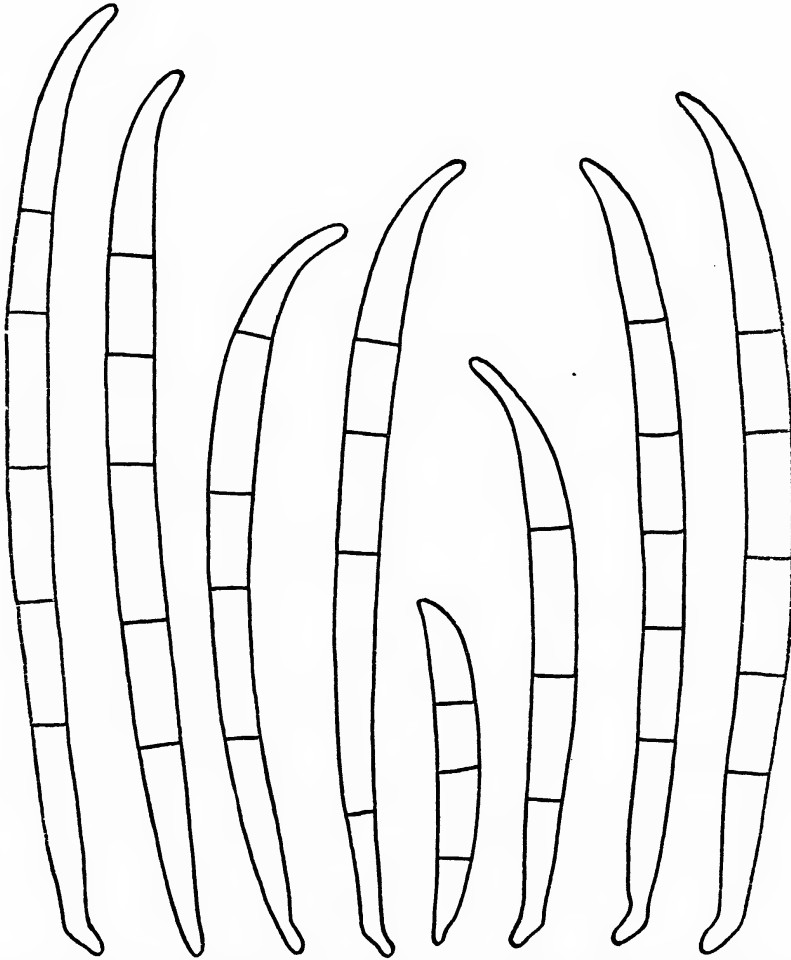


FIG. 4.—Macroconidia of strain Y 29 from sporodochium of a culture 66 days old. X 1733

sibility of a perfect stage. This led to constant examination of cultures for such a stage and to considerable experimentation in an effort to produce it. Various media, low and high temperatures, alternation of low and high temperatures, and other environmental variations were tried in culturing the fungus, but all failed to produce such a stage.

In September of 1922 perithecia appeared in a tube in which both Y 15 and Y 29 had been planted. The Y 15 had been placed at the top of the slant and the Y 29 at the bottom, and the perithecia developed along the line of contact of the two. The same thing occurred in the other of the two tubes similarly inoculated.

In an experiment started at that time a majority of 130 Petri dishes and tubes planted with both Y 15 and Y 29 produced perithecia (Pl. 1, A, B, C), while none of the 59 planted with Y 15 alone, and none of the 62 planted with Y 29 alone,

produced perithecia. In later experiments, perithecia have been found only in tubes or Petri dishes planted with Y 15 and Y 29 or with Y 15 and some other strain, but the yield has been smaller.

PERITHECIA

Macroscopically the perithecia are very similar to those of *Gibberella saubinetii*. Possibly this explains the fact that they have not been reported on corn. Microscopically there is not the least difficulty in distinguishing the ascospores of the two. The straight, usually 1-septate ascospores of the moniliform fungus, so constricted normally at the septa as to give the spore a swollen appearance (Pl. 2, C, D; fig. 5), contrast sharply with the curved, 3-septate ascospores of *G. saubinetii*, normally not constricted at the septa.

Ten representative ascospores from each of the substrata noted in Table III were measured and the averages of these measurements are given in Table III. It will be noted, as previously mentioned, that most of the ascospores are 1-septate.

TABLE III.—Average sizes of 1-, 2-, and 3-septate ascospores, respectively, from cultures in each of which both strain Y 15 and strain Y 29 had been planted, and percentages of the 1-, 2-, and 3-septate ascospores, respectively, from the different substrata

Age of culture	Substratum	Average sizes and percentages of each group in the total number—					
		1-septate		2-septate		3-septate	
		Per cent	Microns	Per cent	Microns	Per cent	Microns
Days							
61	Oat agar		4. 54 by 15. 00				4. 62 by 17. 32
55	Sterilized wheat head		4. 82 by 15. 83				None seen.
55	Oat agar		4. 40 by 15. 32				4. 43 by 16.04
54	Sterilized wheat head		4. 51 by 15. 55				None seen.
55	Potato-dextrose agar	52	3. 95 by 14. 47	21	4. 08 by 14. 78	27	4. 31 by 15. 36
91	Sterilized wheat head	86	4. 35 by 15. 86	9	4. 01 by 14. 78	5	4. 13 by 16. 90
75	Potato-dextrose agar	69	4. 38 by 15. 47	15	4. 38 by 17. 40	16	4. 32 by 17. 40
74	do	61		17		22	
73	do	97	4. 31 by 17. 78			3	4. 42 by 18. 53
	Average		4. 41 by 15. 66		4. 16 by 15. 65		4. 37 by 16. 93

CONIDIAL CULTURES FROM ASCOSPORES

Single germinating ascospores, sufficiently isolated in poured plates so that they could be removed singly, were transplanted to agar slants in test tubes. Such a germinating ascospore, photographed through the bottom of the Petri dish before transferring, is shown in Plate 2, A. This colony transferred, became Fusarium strain A<sub>1</sub>. A similar colony is shown in Plate 2, B. Still another similar colony from a 1-septate ascospore was isolated in a similar way and became Fusarium strain A<sub>2</sub>.

While these Fusarium strains agreed in general with strains Y 15 and Y 29, including the production of microspores in moniliform chains, they have not shown the tendency of Y 29 to produce sporodochia. In form the macroconidia of strains A<sub>1</sub> and A<sub>2</sub> (fig. 6) are very similar to those of strains Y 15 and Y 29 (fig. 2 to 4). Ten representative macroconidia from strains A<sub>1</sub> and A<sub>2</sub> from each of the sources as noted in Table IV were measured and the averages are given. It will be noted that likewise in size the macroconidia of strains A<sub>1</sub> and A<sub>2</sub> are very similar to those of strains Y15 and Y 29 but that the former tend to average somewhat longer, except in the case of the 5-septate conidia of strain Y 29.

The blue-black globoid bodies described for strain Y 15 have been found occasionally in strain A<sub>2</sub>. Perithecia have been produced in tubes in which strains A<sub>1</sub> and A<sub>2</sub> were planted together. They have not appeared in plantings of strain A<sub>1</sub> alone or of strain A<sub>2</sub> alone.

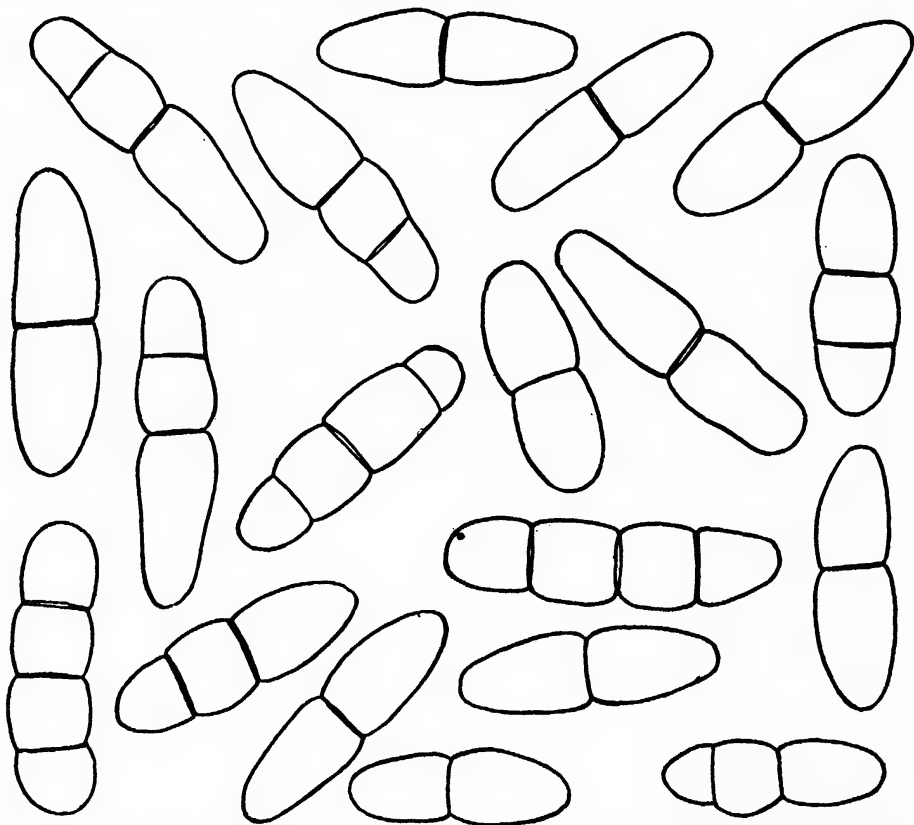


FIG. 5.—Ascospores from a potato-dextrose agar culture 67 days old, planted with both conidial strains Y 15 and Y 29. X 1733

TABLE IV.—Average sizes of 3-, 4-, 5-, and 6-septate macroconidia, respectively, of strains A<sub>1</sub> and A<sub>2</sub> and percentages of each, from different sources

Strain	Age of culture	Source	Average sizes and percentages of each group in the total number—							
			3-septate		4-septate		5-septate		6-septate	
			P. ct.	Microns	P. ct.	Microns	P. ct.	Microns	P. ct.	Microns
A <sub>1</sub>	32	Pseudopionnotes.	29	3.32 by 43.89	1	<sup>a</sup> 3.24 by 44.66	70	3.38 by 53.50	-----	0
A <sub>1</sub>	41	-----	59	3.28 by 41.42	24	3.38 by 47.89	15	3.28 by 55.61	-----	0
A <sub>1</sub>	42	Sporodochium.	26	3.00 by 41.50	-----	-----	74	3.00 by 52.12	-----	<sup>b</sup> 0
A <sub>2</sub>	42	Pseudopionnotes.	70	3.30 by 31.70	5	<sup>a</sup> 3.30 by 42.49	25	3.23 by 48.30	-----	0
A <sub>2</sub>	42	Sporodochium developed on wounded mycelium.	13	3.00 by 42.81	4	3.14 by 47.89	83	3.00 by 58.05	-----	<sup>c</sup> 3.00 by 72.35
A <sub>2</sub>	54	Sporodochium.	32	3.23 by 38.83	-----	-----	68	3.29 by 51.86	-----	<sup>d</sup> 3.00 by 64.68
		Average	-----	3.19 by 40.02	-----	-----	-----	3.19 by 53.24	-----	-----

<sup>a</sup> Three measured.

<sup>b</sup> Rare.

<sup>c</sup> Two measured.

<sup>d</sup> One measured.

There is as yet insufficient evidence to explain why perithecia have been obtained only where two strains have been planted together. Heterothallism is the first interpretation to present itself. Another possibility is that it is a case of chemical stimulation of one strain by the other. As bearing on the latter possi-

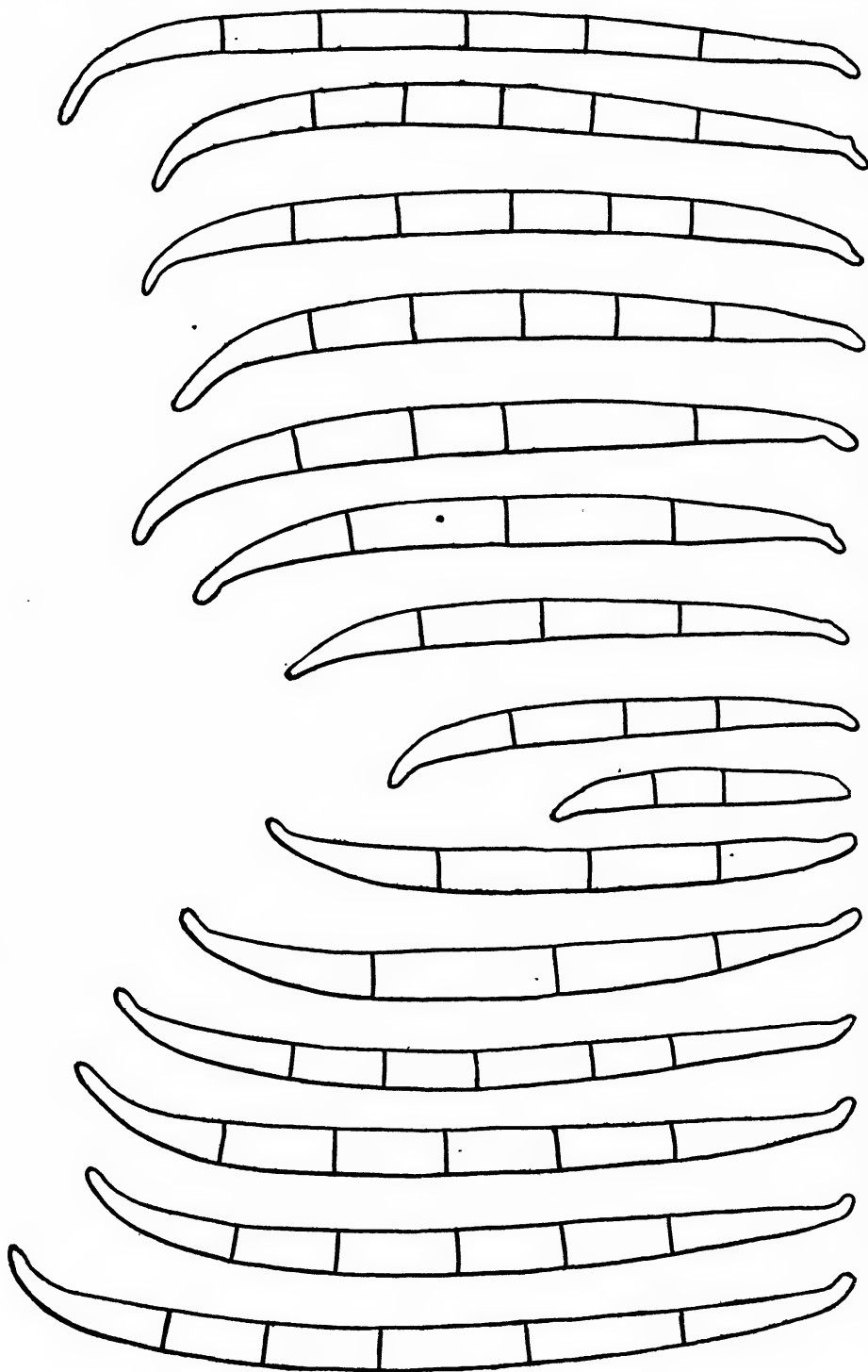


FIG. 6.—Macroconidia of strain A<sub>2</sub> from pseudopionnotes of a culture, 42 days old, derived from an ascospore. X 1733

bility, Heald and Pool (10) report that *Fusarium moniliforme* produces metabolic products which have such a stimulating effect on an entirely different fungus. They grew it with *Melanospora pampeana* Speg. with the result that the latter fungus was stimulated to produce perithecia. It is hoped that investigations in progress may yield contributions along these lines.

## NOMENCLATURE AND SYNONYMY

From the evidence previously presented it seems reasonable to consider the variations in conidial cultures of strains Y 15, Y 29, A<sub>1</sub> and A<sub>2</sub> within the range of variations in a species of this group. While each of these conidial strains differs slightly from *Fusarium moniliforme* as originally described by Sheldon (23), all agree in having moniliform microconidia and 3- to 5-septate macroconidia of the same general type. The evidence is clear that an ascigerous stage has been produced in culture by permitting conidial strains Y 15 and Y 29 to grow together.

The problem, then, is to choose a binomial to meet the situation, both from the standpoint of the ascigerous stage and from that of the conidial stage.

## ASCIGEROUS STAGE

The first attempt to solve the problem was an effort to find some previously described species of *Gibberella* with which the perfect stage might be placed. *Gibberella acervalis* seemed to be the only one to consider seriously. This species was suggested by a statement of Holbert and Hoffer (12) that *G. acervalis* (Moug.) Wr. non Sacc. was associated with root-, stalk-, and ear-rots of corn, and the fact that a culture received from them was found to have microconidia in chains and macroconidia of the *Fusarium moniliforme* type.

*Gibberella acervalis* (Moug.) Wr. non Sacc. is figured by Wollenweber (29, Pl. 2 to 4). No complete description under this name has been found. Only ascospores and macroconidia are included by Wollenweber. The ascospores are 1-septate with limits of measurements 4.5 to 5.5 by 12 to 17 $\mu$ . Macroconidia are pictured on two of the three plates devoted to *G. acervalis*. Those on Plate 2 are 3-septate, 2.75 to 3.25 by 25 to 38 $\mu$ . Those on Plate 3 are 3- (3 to 5-) septate, 2.25 to 3 by 30 to 50 $\mu$ . On both plates Wollenweber comments that the relation of the conidia to the perfect stage is doubtful, but on Plate 2 a peculiarity in the Latin construction suggests that the intended meaning may have been to state that there was no doubt as to the genetic relationship. The fungus is reported as having been found only on wood, such as branches of alder, roots of oak, branches of willow, and juniper.

The size and form of the ascospores and macroconidia described by Wollenweber correspond rather closely with those of the fungus under consideration in this paper. However, no 3-septate ascospores are mentioned, and no microconidia, to say nothing of microconidia in chains, and the substrata for the two are widely different.

*Gibberella acervalis* (Moug.) Sacc. (21 p. 318) seems no more likely to be the perfect stage of the corn fungus than *G. acervalis* (Moug.) Wr. It is described as having only 3-septate ascospores, 6 by 8 $\mu$ , and includes a spermogonial stage with spermogonia about a third as large as the perithecia, and spermatia very small, cylindrical, and oscillatory. It also is reported as having been found on wood.

It would seem, therefore, that neither *Gibberella acervalis* (Moug.) Wr. nor *G. acervalis* (Moug.) Sacc. covers the present case.

## CONIDIAL STAGE

Norton and Chen (15) call attention to the fact that the fungus *Oospora verticilloides* Sacc. is very similar to or identical with Sheldon's (23) *Fusarium moniliforme*. They use the name *O. verticilloides* for the fungus from sweetcorn seed with which they were working, and add that if it should produce *Fusarium* macroconidia it would be identical with *F. moniliforme* Sheldon, but that the logical name for the fungus would be *Fusarium verticilloides*. Cultures kindly supplied by Norton and Chen produced *Fusarium* macroconidia when grown on oat agar in the present investigations.

Likewise, Manns and Adams (14) state that—

The fungus *Oospora verticilloides* described by Saccardo on corn is no doubt identical with *Fusarium moniliforme*.

*Oospora verticilloides* Sacc. (20, v. 2, p. 546) has been discussed by European writers chiefly in its supposed etiological relation to pellagra. There is nothing about the original description or the illustration which disagrees with the microconidia of *Fusarium moniliforme*, but the description is somewhat lacking in detail. A translation of the description is as follows: Colonies white, conidiophores simple, branched or subverticillate, conidia in short chains, and oblong-fusoid or sub-obclavate, 2.5 to 3 by 8 to 10 $\mu$ , hyaline, found on broken corn kernels.

Cuboni (2), working on pellagra in Italy, in 1882, reported a fungus almost universally present in corn of both good and poor quality in the pellagra districts, which he referred provisionally to Saccardo's *Oospora verticilloides*. He based the identification on Saccardo's illustrations, as he did not have access to the description. Cuboni's description agrees with that of Saccardo, except that he does not mention the catenulate arrangement of conidia. His three figures, showing mycelium, microconidia, and germinating microconidia, add nothing to his description.

Deckenbach published articles on *Oospora verticilloides* in connection with pellagra in Bessarabia, in 1896 (3), 1899 (4, 5), and 1907 (6). The 1907 article is a summary and contains all his data on the fungus. He gives a more detailed account of it than Cuboni and reports it as a widespread parasite of corn everywhere in Bessarabia. It reaches the individual ovaries of the corn by following the silk before the milk stage. He speaks of the scattered infected kernels with their broken pericarps and crumbling white contents of starch grains and fungus spores standing out sharply against the amber-gold of the normal kernels. He mentions culturing it on agar, potatoes, beef-peptone-gelatin, sterilized corn, and sterilized corn meal. Growth on the first three media mentioned is light. The gelatin is liquefied by the fungus. The color on the corn and corn meal changes from white to pink in three or four weeks and finally intensifies to violet in six to eight weeks.

Tiraboschi (26), in 1905, also concerned with the cause of pellagra in Italy, gives a very careful and detailed description of *Oospora verticilloides*. The optimum temperature for the fungus he finds to be 25° to 30° C. He gives certain cultural characters on the following media: Potatoes moistened with Raulin's fluid, Raulin's fluid agar, broth, gelatin, corn decoction, and milk. He studied the formation of the chains of microconidia as it occurred around the margin of hanging-drop cultures.

Not all of the cultural methods of Tiraboschi have been duplicated by the writer with *Fusarium moniliforme*. There is nothing particularly distinctive about the cultural characters he mentions, and in general they are what one would expect of *F. moniliforme*. The morphological characters given for the fungus with which he worked are very detailed and agree perfectly with *F. moniliforme*. He even mentions the fact that the last formed conidium in the chain is shorter and more nearly round than those formed first, and that the proximal ends of the conidia are more acute than the distal ends. His drawings are careful and might well be those of *F. moniliforme*.

In addition to this, the specimens of corn kernels attacked by *Oospora verticilloides* Sacc. in D. Saccardo's *Mycotheca italica* as specimen No. 1372 collected at Udine, Italy, by Prof. Sbozzi in 1903, are very similar to the numerous specimens of attacked corn kernels examined in the present investigations. The lesions on the kernels of the Saccardo specimen, that is, the blisters and cracks, are identical with kernel lesions in which *Fusarium moniliforme* Sheldon is commonly found in this country. Microscopic examination of the Saccardo material showed

mycelium but no conidia. The mycelium is like that of *F. moniliforme* but might equally well be some other fungus.

Reviewing the data, it seems that only evidence of the presence of macroconidia in *Oospora verticilloides* is necessary to establish its identity with *Fusarium moniliforme*.

Cuboni (2) states that occasionally he found a spore with two or three divisions, but there is nothing in his drawings to indicate macroconidia. No other reference to septate spores has been found.

To those who have worked with *Fusarium moniliforme*, this discrepancy will not appear impossible of explanation. The macroconidia found on corn are very few, so few ordinarily that they might not be found at all with examinations of abundant material, or probably would be regarded as spores of some other fungus. The variability of cultures in their tendencies to produce macroconidia is well known, and the media used by many of the investigators were not conducive to the production of macroconidia.

Since the evidence is so strongly in favor of *Oospora verticilloides* Sacc. and *Fusarium moniliforme* Sheldon being identical it seems unfortunate that the lack of description of macroconidia for the former species or an opportunity to compare authentic cultures of the two, makes it impossible to decide the case definitely.

Tiraboschi (26) considers the matter of synonymy and concludes that *Oospora verticilloides*, *O. hyalinula* Sacc., and *O. candidula* Sacc. are identical. He obtained specimens of *O. verticilloides* and *O. hyalinula* from Saccardo, and grew them and the fungus he, himself, had identified as *O. verticilloides* in parallel series on all the media he used. He states that he could not tell one from the other. He was not able to obtain a specimen of *O. candidula*, but notes that Saccardo pictures it with a branched chain of conidia, a condition never found in the fungus with which he was working. He includes it in synonymy, however, because Saccardo's description of it agrees more closely with his fungus than Saccardo's description of either *O. verticilloides* or *O. hyalinula*. He also notes the fact that the name *O. hyalinula* antedates *O. verticilloides*, but chooses the latter name because it was established by the more complete description. *Oospora hyalinula* was originally described as *Torula hyalinula* Sacc. (20, v. 1, p. 265), the transfer to *Oospora* being made by Penzig (16, p. 453) for Saccardo. To these synonyms he adds the following list, doubtful not because their descriptions disagree with that of *O. verticilloides*, but because they are inadequate: *O. dubiosa* (Speg.) Sacc. and Vogl. ? *O. alba* (Preuss.) Sacc. and Vogl. ? *O. ovalispora* (Berk.) Sacc. and Vogl. ? *O. circinans* (Bon.) Sacc. and Vogl. ? *O. epilobi* (Cord.) Sacc. and Vogl. ? *O. hypoxylicola* (Preuss) Sacc. and Vogl. ? *O. nectricola* Rich. ?

Obviously the only way in which the relations of these various organisms can be satisfactorily determined is by comparing authentic cultures of them, a method not practicable for the present, at least. It does seem reasonable, however, to regard *O. hyalinula* as a synonym of *O. verticilloides* on the basis of the comparison of authentic cultures of them made by Tiraboschi.

Even the taxonomic meaning of Sheldon's *Fusarium moniliforme* is doubtful from the standpoint of the fusariologist. Sherbakoff (25) has stated that he believes there are several distinct species among those answering Sheldon's description of *F. moniliforme* and has established the new section, "Moniliform," for the group with the following characters:

Macroconidia of intermediate Roseum-Elegans type, with very thin walls, mostly 3-septate; microconidia also in chains; no chlamydospores; color of substratum from none to violet.

Differences in the cultures studied in the present investigations might confirm this view. However, the following observations also have been made. Certain



strains that produced macroconidia in abundance at first lost this character after a time and afterwards produced nothing but mycelium and microconidia. Loss of color seemed to accompany this change in many cases. In a few instances cultures never produced pseudopionnotes or sporodochia, and showed very little color.

Loss of tendency to produce macroconidia in culture may be explained as due to growth under unfavorable conditions, or repeated transferring of mycelium or microconidia. Whether cultures that never produced sporodochia even from the time of isolation are to be interpreted as having been under unfavorable conditions in nature, or are to be considered species distinct from those that do produce sporodochia is not clear. On the whole it seems that insufficient evidence is at hand at present to justify the making of a number of new species. It seems preferable to retain the group under a single specific name with the understanding that a certain degree of variability is included.

Summarizing the situation, there can be no doubt as to the identity of *Fusarium moniliforme* Sheldon, when the name is used in the sense just defined and the conidial forms considered in this investigation. There is very little doubt as to the identity of *Oospora verticilloides* Sacc. with these forms, but the lack of a description or even definite mention of macroconidia in literature for *O. verticilloides*, and the lack of an opportunity to study an authentic culture of it, necessitate questioning the synonymy. *Oospora hyalinula* Sacc. is placed on an equal footing with *O. verticilloides* because of Tiraboschi's conclusion that they were identical after studying authentic cultures of them, as previously explained.

Thus only *Fusarium moniliforme* Sheldon remains to be considered if a conidial name is to be used for the new combination. This name seems particularly desirable in that it is the one best known to plant pathologists and will cause the least confusion in a new combination. The proposed new name and synonyms follow.

#### TECHNICAL DESCRIPTION

**Gibberella moniliformis** (Sheldon) n. comb.

? *Torula hyalinula* Sacc. 1878, Micothecae Venetae no. 1255; *Michelia* 1: 265; *Fungi italici delineati* no. 878.

? *Oospora hyalinula* Sacc. 1882, Penzig, *Michelia* 2: 453.

? *Oospora verticilloides* Sacc. 1882, *Michelia* 2: 546. *Fungi italici delineati* no. 879.

*Fusarium moniliforme* Sheldon, 1904, Nebr. Agr. Exp. Sta. Ann. Rpt. 17: 23-32, illus.

**PERITHECIAL STAGE.**—Perithecia scattered or gregarious, ovoid to sub-conical, free on the surface of the medium or embedded in mycelium, or in a tubercular plectenchymatic stroma, ostiolate, rarely 2-ostiolate, 225 to 300 by 300 to 375  $\mu$ ; peridium of cells fairly uniform in size, and fairly smooth, blue-black when viewed macroscopically, dark blue by transmitted light; no paraphyses observed; ascospores 8, arranged irregularly in two rows, practically straight, fusiform to ellipsoid, rounded at the ends, often constricted at the septa, pale ochraceous salmon in masses, 1 to 3 septate, the 1-septate predominating, 3.9 to 4.8 by 15 to 19  $\mu$ .

**CONIDIAL STAGE.**—Microconidia produced basipetally on simple to verticillate conidiophores, adhering to form chains, or slipping aside to form balls or clusters at the ends of the conidiophores, obovoid to ellipsoid, 2 to 3.5 by 5 to 10  $\mu$ ; macroconidia somewhat curved especially near the apex, gradually attenuate toward apex, pedicellate, borne on aerial mycelium, in pseudopionnotes or sporodochia, 3- to 5-septate; 3-septate conidia 2.9 to 3.2 by 32 to 40  $\mu$ ; 5-septate conidia 3.0 to 3.2 by 44 to 56  $\mu$ ; pseudopionnotes and sporodochia, pale ochraceous-salmon, vinaceous-cinnamon, or pale purplish vinaceous (19); mycelium dense, medium high, wooly, showing such colors as sea-shell pink, pale vinaceous fawn, pale purplish vinaceous, pale vinaceous-pink, purplish vinaceous, deep livid brown, pallid mouse gray, plumbago gray (19), substratum showing such colors as grayish olive, purplish gray, dark plumbago gray, vinaceous slate, dark vinaceous-drab, cinnamon drab and Indian purple (19).

**HABITAT.**—Perithecial stage known only in culture. Conidial stage very commonly associated with corn plants, as a saprophyte or parasite. Reported as a weak parasite in damping-off pine seedlings (18), as a cause of rootrot of older pines (9), and as having been isolated from rotted potato tubers (29). Probably a widespread saprophyte.

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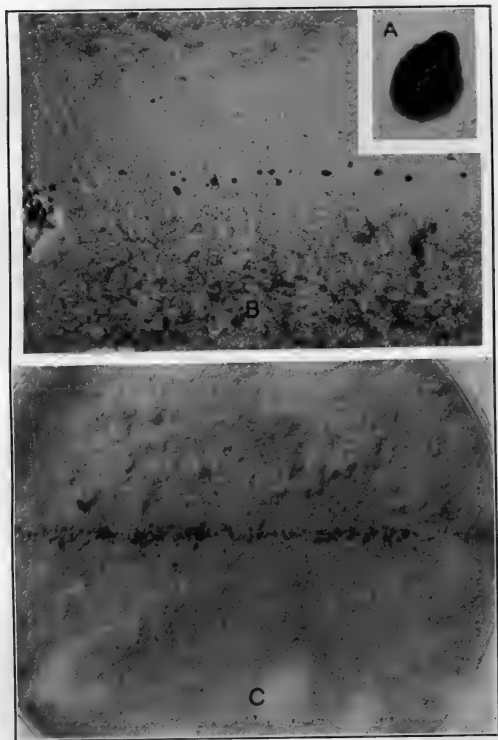
## PLATE 1

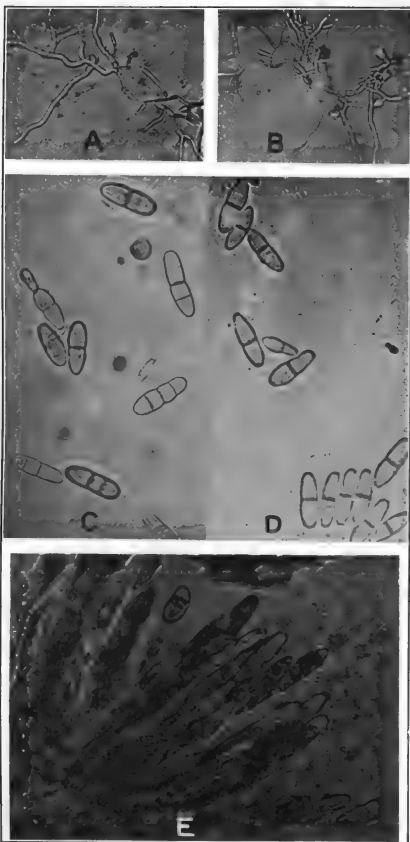
Photomicrographs of *Gibberella moniliformis*

A.—Perithecium.

B.—Row of perithecia along the line of contact of Y 15 and Y 29 planted in a Petri dish on bean agar.

C.—Row of perithecia along the line of contact of Y 15 and Y 29 planted in a Petri dish on oat agar.





## PLATE 2

### Photomicrographs of *Gibberella moniliformis*

- A.—Colony developing from a 2-septate ascospore. X 190.
- B.—Colony developing from a 1-septate ascospore. X 190.
- C, D.—Ascospores. X 750.
- E.—Ascospores in asci. X 750.

# EXPULSION OF AECIDIOSPORES BY THE MAYAPPLE RUST, *PUCCINIA PODOPHYLLI* SCHW<sup>1</sup>

By B. O. DODGE

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The mechanics of spore discharge from an aecidium was recently discussed in a short account of the formation of germ pores of aecidiospores of *Gymnosporangium myricatum*.<sup>2</sup> The aecidiospore of the bayberry rust is provided with germ pores, six or seven in most cases. The writer found that, at the point where a germ pore is to be developed, there is a thickening of the spore wall over a small area in such a way that there is formed a little ball-like body, or "pore plug," which is separated from the rest of the spore wall. The germ pore becomes evident as soon as the plug is dislodged. By studying carefully stained sections of young aecidia it may be seen that the plug or pore formation begins with about the fourth spore in the chain and is completed when the spore reaches maturity. Seeing the spores densely packed in an aecidium, and with the spore walls deeply indented by the plugs, one readily understands how the plugs function in providing a more effective mechanism of spore discharge. Although the aecidiospore of the long-cycled orange-rust, *Gymnoconia*, on *Rubus*, does not form such pore plugs, still the spore is discharged with considerable force, proving that the plug, though serving a useful purpose in this connection, must act only in an accessory capacity in dislodging the spores in case of the bayberry rust. In view of the fact that such bodies have not been reported by those investigators who have studied the cytology of the rusts, it was thought likely that the persistence of that part of the spore wall as a little plug or ball filling or covering the germ pore was rather an unusual occurrence. The writer has found a second species of rust where pore formation is accompanied by the development of similar bodies which persist as little plugs and no doubt take part in forcibly discharging the spores from the aecidium.

## THE MAYAPPLE RUST, *PUCCINIA PODOPHYLLI*

The aecidia of *Puccinia podophylli* frequently cover large areas of the leaf so that the normal functioning of the lower epidermis must be seriously interfered with. The orange-rusts of *Rubus* are perennial. It was pointed out by the writer<sup>3</sup> that the presence of the gametophytic mycelium so stimulates the host that large numbers of stomata are formed on the upper side of the leaf where normally there are none. A similar study of *Podophyllum* leaves heavily infected with the mayapple rust sufficed to show that no such an effect follows the invasion of a leaf in this case. Stomata were rarely found on the upper side of the leaf, whether infected or not. Although the results of this study were disappointing, some further information was obtained relative to the discharge of the aecidiospores.

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<sup>1</sup> Received for publication July 23, 1924—issued Nov., 1924.

<sup>2</sup> DODGE, B. O. AECIDIOSPORE DISCHARGE AS RELATED TO THE CHARACTER OF THE SPORE WALL. *Jour. Agr. Research* 27: 749-765, illus. 1924.

<sup>3</sup> DODGE, B. O.—EFFECT OF THE ORANGE-RUSTS OF *RUBUS* ON THE DEVELOPMENT AND DISTRIBUTION OF STOMATA. *Jour. Agr. Research* 25: 495-500, illus. 1923.



It was noticed that where leaves were left in damp chambers overnight, aecidiospores would be found dusted over areas considerably beyond the margin bearing sori as though the spores had been discharged from the aecidia with some force. In order to prove whether this was a fact, the following simple method was employed. Petri dishes about 2 cm. in depth, containing a small amount of agar to maintain the desired humidity, were prepared. Small squares of the leaf bearing aecidia were set up against the side of the dish (fig. 1, F.) in such a way that should the spores be discharged violently, they would be shot out and come to rest on the agar. Within a few minutes after the apparatus was set up, it was found that a number of spores had already been discharged, some of them coming to rest fully a centimeter and a half from the point of discharge.

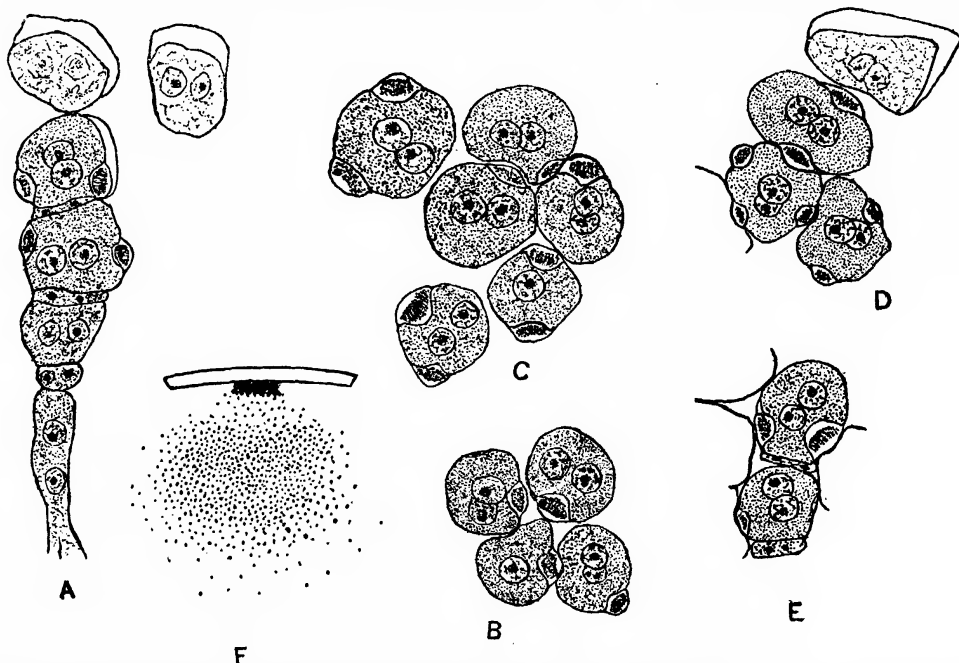


FIG. 1.—Aecidiospores of *Puccinia podophylli* Schw. (A), A chain of young spores showing the basal cell surmounted by three spores and a peridial cell. The first spore shows no local thickenings of the wall. The other two spores show two pore plugs. No plugs are developed in the walls of the peridial cells. (B) Four spores with one pore in each similarly located showing in this section. (C) Large spores with exceptionally large pore plugs. (D) Oblique section; four plugs in one spore. (E) Deep indentations in upper spore from pressure due to the development of the plugs and not to shrinkage in fixation. (F) Spore print of aecidiospores on agar. A small piece of leaf was fastened against the side of the Petri dish. As the spores were discharged they came to rest on the agar

After 18 hours, a definite spore print had been laid down. The largest number of spores had come to rest somewhat over one-half a centimeter away from the aecidia discharging them. During a number of trials, it was found that very few spores were shot beyond a distance of  $1\frac{1}{2}$  cm. In order to find out how high the spores could be shot vertically, pieces of the leaves bearing aecidia were laid on the agar in the bottom of the dish so that spores shot upward would strike the cover. To insure that the spores would stick to the cover, a thin layer of agar was spread over its surface. Very few spores were found on the cover when it was placed 1 cm. above the leaf.

Dr. A. H. R. Buller<sup>4</sup> has written the author that he believed a light object like an aecidiospore would be projected vertically just about as high as it would

<sup>4</sup> The reader will find an interesting account of experiments with the "toy balloon-gun" illustrating spore discharge in the following book: BULLER, A. H. R. RESEARCHES ON FUNGI. 2: 33-38. New York. etc. 1922.

be shot horizontally when projected from the aecidium. In other words, if the same force is applied to shoot a toy balloon upward vertically as is applied to throw it horizontally, the distance traversed by the balloon before it begins to fall in a straight line is about the same in both cases.

As only a few spores in each experiment were found at a distance of  $1\frac{1}{2}$  cm. from the aecidia, this may be assumed to be about the maximum horizontal distance that the spores could be thrown under these conditions. In the limited number of trials made to determine how high the spores could be shot the maximum distance seems to have been about 1 cm. No doubt if the experiments had been made with the rust on leaves attached to the plant the results would have been somewhat different.

#### SPORE DISCHARGE FROM AECIDIA OF GYMNOCONIA INTERSTITIALIS

Similar experiments were performed with orange-rust aecidia on leaves of *Rubus*. When small pieces of leaves bearing aecidia were employed the spores were shot vertically about 1 cm. but when entire leaves were used the vertical distance covered was somewhat greater, 1.3 cm. in some cases. Entire leaves were not used to determine the maximum horizontal distance. Using small squares of infected leaves, it was found that the maximum distance was about 1.1 cm. It makes a great deal of difference which orange-rust of *Rubus* is used in these experiments. The spores of the short-cycled form are rather waxy and tend to cling together so that they pile up in the sorus. Occasionally some of the spores are set free singly with a small amount of force so that they are found a few millimeters from their aecidia. On the other hand, when the long-cycled orange-rust is used a dusty spore print is laid down.

#### PORE FORMATION IN AECIDIOSPORES OF PUCCINIA PODOPHYLLI

The first thing that particularly attracts attention in the examination of a spore print of the *Podophyllum* rust under the microscope is the large number of little bodies which lie around on the agar among the spores and attached to them. (Pl. 1.) These bodies resemble closely the "pore plugs" found attached to the spores of the bayberry rust previously mentioned. In order to prove whether or not they had the same origin, it was found necessary to study sections of young aecidia. Small pieces of leaf which showed the first signs of aecidial growth were fixed in Flemming's weaker fluid. Sections were stained with the triple stain.<sup>5</sup> In case of the *Myrica* rust the first indications of pore formation is seen in connection with the fourth or fifth spore in the chain. The process certainly begins earlier with the *Podophyllum* rust, for the second spore in the chain frequently shows one or two places where the wall is being disorganized around a thickened portion which takes the gentian violet stain. (Fig. 1, A.) The older the spore the larger the plugs become. If there are six or seven spores in the chain the plugs in the upper ones are so large as to indent deeply the walls of adjacent spores. In stained preparations the plug appears to lie in a cavity somewhat larger than the plug. There is no doubt that there is a certain amount of mucilaginous disorganization accompanying pore formation which also adds to the pressure, tending to spring back, or indent, the spore walls. The peridial cells at the upper end of a chain of spores never show germ pores or pore plugs. (Fig. 1, A.)

In favorably oriented sections three or four germ pores can be seen in one spore. (Fig. 1, D.) Exceptionally large pore plugs are shown in figure 1, C.

<sup>5</sup> Miss Ruth Colvin prepared the slides for this study and made the photographs which are reproduced in this paper.

As the spores mature and are discharged, the plugs appear to become rounded. (Pl. 1.) Some of them are dislodged during flight, others fall off as the spore comes to rest. One or two plugs may cling to the spore. Herbarium specimens of this rust show these bodies among the spores and they are undoubtedly always developed. Any object which could cause such indentations in elastic spore walls as are shown in figure 1, C, must certainly serve as a good fulcrum against which the spore wall can react as the spore breaks loose from its neighbors.

Dr. Buller has called the writer's attention, in an advance copy of a section which he has written for his new book on the fungi, to the work of Zalewski<sup>6</sup> on aecidiospore discharge. Zalewski found that in the case of four species of rusts the aecidiospores were discharged violently to a distance amounting in some instances to 1.5 to 2 cm. He also found that the spores were shot vertically to a height of 1.0 to 1.5 cm. This author gave no explanation of the phenomenon, and offered no suggestion as to the mechanics of the process.

### SUMMARY

A study was made of the distribution of stomata on leaves of *Podophyllum* infected with the *Puccinia podophylli*. The host is not stimulated to develop additional stomata.

The germ pores of aecidiospores of this rust are developed as the result of localized thickenings of the wall in such a way that little plugs are formed and these become separated from the rest of the wall. The plugs, deeply indenting the elastic walls, serve as fulcrums against which the walls react as the spore is set free, so that it is discharged with considerable violence.

Persistent pore plugs are not developed by the aecidiospores of the orange rusts of *Rubus*. The spores of the long-cycled *Gymnoconia* are nevertheless discharged with some violence. Those of *Caecoma nitens*, being more waxy, tend to cling together and remain in the sorus, where they may germinate.

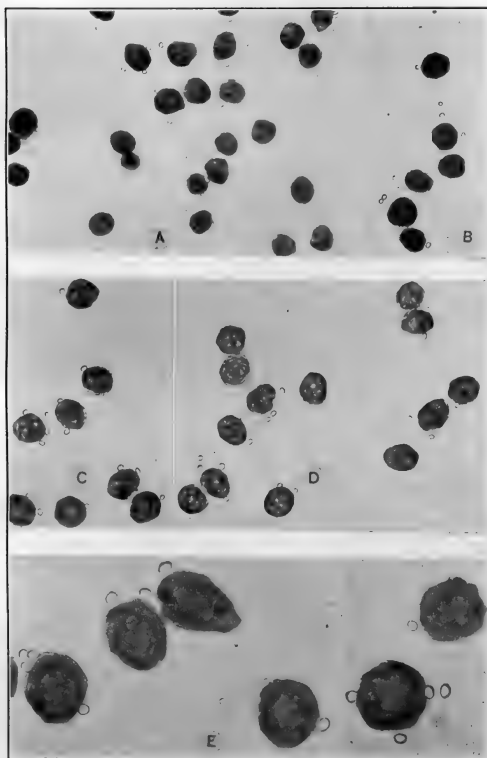
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<sup>6</sup> ZALEWSKI, A.—Ueber sporenabschnurung und sporenabfallen bei pilzen. *Flora* 66: 228-234, 249-271. 1883.

## PLATE 1

### *Puccinia podophylli* Schw.

The aecidiospores were photographed as they had fallen on the agar after being shot horizontally about one centimeter. The pore plugs appear in the pictures as little circles, some of them still attached to the spores. A to D.—Photographed using an 8 mm. objective and  $\times 10$  Oc. E.—More highly magnified.



# THE HEAT OF WETTING OF SOIL COLLOIDS<sup>1</sup>

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## INTRODUCTION

The property of liberating heat on being wetted is well known to be characteristic of most colloidal substances and is generally thought to be essentially a function of surface. It was shown in a qualitative way over a century ago that certain powders liberate heat when wetted by liquids (12).<sup>2</sup> Soils and clays were included among the powders studied by some of the early investigators, who noted marked increases in temperature when the dry material was wetted by water and other liquids (15). Mitscherlich (6) was the first investigator to make a thorough study of the heat of wetting of soils. He used soils of different texture and varied the initial water content in a series of determinations. He was able to show a close relationship between heat of wetting and hygroscopicity, both of which he regarded as a measure of surface. He regarded the heat of wetting as a function of the colloidal material but considered the results as being of qualitative significance only, since the heat evolved probably depended upon the nature as well as upon the quantity of colloid present. Bouyoucos (2), however, has recently suggested that heats of wetting of soils should serve as a basis for the quantitative estimation of their colloid content. It is important in this connection to ascertain whether the colloidal material in different soils varies in heat of wetting.

Heat of wetting has been assumed by different investigators to be essentially a function of surface. The older concepts assumed that the effective surface could be determined microscopically (9). However, it is now recognized that this is impossible because of the submicroscopic size of many of the primary particles in substances having high heats of wetting. In the case of silica gel, Patrick and Grimm (10) have recently suggested that the surface influencing the heat of wetting is that of a water film surrounding silica nuclei which are approximately  $5\ \mu\text{m}$  in diameter. The influence of surface of some kind upon colloidal properties is generally recognized. It is important, therefore, to know the heat of wetting of colloidal material from different soils, aside from any possibility that the determination could be used as a measure of the quantity of colloid in soils.

## EXPERIMENTAL PROCEDURE

The method used for the determination of heat of wetting was very similar to that used by Patrick and Grimm (10) in their study of the heat of wetting of silica gel. The calorimeter consisted of a 150-cc. Dewar flask fitted with a fiber cap through which was inserted a small stirrer, a thermometer graduated to  $0.025^\circ\text{C}$ ., and a short piece of No. 36 therlo wire, with copper leads, to serve as a heating coil. The whole apparatus was placed in a large air thermostat regulated to  $25^\circ$ .

The water equivalent of the calorimeter was determined by passing a measured electrical current through a heating coil of known resistance for 5 minutes and

<sup>1</sup> Received for publication July 2, 1924—issued Nov., 1924.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 935.

noting the rise in temperature. The water equivalent thus included the heating effect of the stirrer and loss due to radiation during the 5-minute period required for determinations. The water equivalent of the calorimeter was found to be 16.8 gm. of water. The specific heat of the soil or the colloid was taken as 0.2 in each case.<sup>3</sup>

A 5 to 15 gm. sample of soil or air-dried colloid was ground to pass a 50-mesh sieve, dried for 18 hours at 110° C., cooled, weighed, and placed in a desiccator in the thermostat, where it was brought to a temperature of 25°. Seventy-five grams of water were placed in the calorimeter and allowed to come to a temperature of 25° ± 0.05°. When necessary a current was passed through the wire for a few seconds in order to reach more quickly the temperature desired. After reaching a temperature of 25°, the calorimeter was allowed to stand in the thermostat for at least 30 minutes before making a determination. It was found that under these conditions the temperature of the water would not vary more than 0.02° over a period of 5 minutes. Having thus reached constant temperature, the sample was added through a copper funnel inserted in a hole in the fiber cap. At the end of 5 minutes, the thermometer reading was taken. The temperature at the end of this time was usually at or near its highest point where it remained with slight recession for several minutes.

Duplicate determinations as a rule showed a maximum variation of about 0.5 calories per gram. This variation was just as great for materials of low heat of wetting as for those with high values. It is thus seen that for the average soil colloid the result is accurate within about 5 per cent; while for those of very low heat value the percentage may run considerably higher.

## HEAT OF WETTING OF COLLOIDAL MATERIAL AND OF SOILS

The colloidal materials studied were prepared by the use of a supercentrifuge as described in a previous publication of this bureau (4). The particular samples used were selected from a list of some 50 which had previously been investigated for adsorptive capacities and chemical composition. They covered very nearly the variations in adsorptive capacity and chemical composition encountered in the 50 samples. It was therefore presumed that they would cover fairly well the range of heat of wetting values to be expected in the colloidal material from normal soils. The soils from which these colloids were extracted were some of the important soil types and differed widely in texture and other characteristics. The heats of wetting in water of the colloidal materials and corresponding soils are shown in Table I.

TABLE I.—*Heat of wetting of colloidal material and corresponding soil*

Type of soil or colloidal material	Heat liberated per gram of colloidal material	Heat liberated per gram of soil
	<i>Calories</i>	<i>Calories</i>
Cecil clay loam subsoil.....	4.5	1.6
Chester loam soil.....	7.2	1.1
Marshall loam soil.....	14.2	4.3
Miami silty clay loam subsoil.....	11.8	6.4
Norfolk fine sandy loam subsoil.....	6.0	1.5
Sassafras silt loam subsoil.....	9.8	2.2
Sharkey clay soil.....	16.3	9.6
Wabash silt loam soil.....	17.6	5.2

<sup>3</sup> Patten (11) found the specific heats of several soils, including sands, a muck and a clay, varied between 0.16 and 0.21. Mitscherlich (7) gives values for sands and clays ranging from 0.13 to 0.27. The heats of wetting of the samples tested would have been affected less than 1.6 per cent by a variation of 0.1 in the specific heat of the sample.

The heats of wetting of soil colloids are shown to be of an order comparable with those of organic and inorganic gels studied by various investigators. Rodewald (14) found 19.4 to 24 calories evolved per gram of starch. Bouyoucos<sup>4</sup> (2) found 9.6 calories per gram for "ferric hydroxide" and 21 calories for "aluminum hydroxide." Various investigators working with different preparations of silica gel have found from 6.9 to 24.2 calories per gram (5, 10).

Table I shows that the heat liberated from the different soil colloids varies widely. For example, the Wabash colloid, which gives over 17 calories per gram, has nearly four times the heat value of the Cecil colloid with 4.5 calories. This difference is of about the same order as the difference in the extreme values for dye and ammonia adsorption.<sup>5</sup> The adsorption of water vapor, however, was much less variable.<sup>6</sup>

It is to be noted also in Table I that there is not much correspondence between the heat of wetting of the colloidal material and of the whole soil from which it was isolated. The heat liberated by the colloidal material was in some cases about twice that of the whole soil and in other cases more than six times the soil value. Certain of the soils gave higher heat values than the isolated colloids from other soils. The Miami, Sharkey, and Wabash soils, for instance, each gave a higher heat of wetting than the colloidal material from the Cecil. The heats of wetting of the different soils would not, therefore, be expected as a rule to show the relative quantities of colloidal material present. However, soils known to contain colloids of similar properties might be compared on the basis of heat of wetting of the whole soil and some idea of the relative colloid content be obtained.

#### HEAT OF WETTING OF NONCOLLOIDAL SOIL PARTICLES

It was shown in a former publication of this bureau (1) that the adsorptive capacity of the soil was associated almost entirely with the colloidal matter, and that the minerals making up the noncolloidal portion possessed but little adsorbing power. Of the common soil minerals, the micas were the only ones to show any significant adsorption. It was thought desirable to ascertain whether or not the heat of wetting of a soil was also practically all due to the colloidal material. The heat of wetting of mineral powders ground to a degree of fineness comparable with that of the minerals in the soil should show whether or not the noncolloidal part of the soil contributes appreciably to the heat of wetting of the whole soil.

For the study of mineral powders, commercial samples of quartz flour and orthoclase were used, all of which passed a 130-mesh sieve and contained much very fine material. Samples of hornblende and muscovite were prepared by grinding the minerals several days in a steel ball mill. These samples also passed a 130-mesh sieve and contained much that was very fine. The quartz, hornblende, and orthoclase each gave a temperature increase on wetting of less than 0.05° C., which was not greater than the probable error. Thus these common soil minerals above colloidal size would not contribute appreciably to the heat of wetting of a soil. Muscovite gave a heat of wetting of 1.3 calories per gram. This quantity is small, but it might constitute a significant part of the heat value of a soil containing a small quantity of colloid and a high percentage of mica. The

<sup>4</sup> These calculations are made on the basis of air dry weight.

<sup>5</sup> Additional data, as yet unpublished, indicate a close relationship between the heat of wetting power of a soil and its capacity to adsorb ammonia.

<sup>6</sup> It was indicated that the approximate constancy of the different colloids for water vapor adsorption probably applied only to an atmosphere whose humidity was similar to that produced by 3 per cent sulphuric acid (by weight). If stronger acid were used the results would be more variable (4, p. 12).



heat of wetting data closely parallel the results obtained in a more extensive study of soil minerals with respect to adsorption (1), indicating that the heat of wetting of a soil is almost entirely a function of the colloidal material.

### HEAT OF WETTING AS A BASIS FOR ESTIMATING THE COLLOIDAL MATERIAL OF SOILS

While the heat of wetting of a soil is doubtless due almost entirely to the colloidal matter present, it is evident from the data given in Table I that the heats of wetting of different soils do not indicate the relative contents of colloidal material. This simple relationship does not obtain because of the wide variation in the heats shown by different colloids. It is possible, however, that the approximate colloidal content of a soil could be estimated from the heat of wetting of the soil if the heat of wetting of the particular kind of colloid present in the soil were known. If a sample of the colloidal material of a soil were extracted and the heat of wetting determined, then the formula

$$\frac{\text{heat of wetting per gram of soil}}{\text{heat of wetting per gram of colloid}} \times 100,$$

should indicate approximately the percentage of colloidal material in the soil. This general method of estimating the colloidal contents of soils from the standpoint of adsorptive capacities has been investigated in this bureau (4, 8, 13).

Table II shows the percentages of colloidal material present in eight soils, as indicated by the ratio of heat of wetting of soil to that of colloid. The data for this calculation are given in Table I. Table II also gives, for comparison, the percentages of colloid in the same soils indicated by adsorption ratios.

TABLE II.—Percentages of colloidal material in soils calculated by heat of wetting ratio and by dye, water, and ammonia adsorption ratios

	Colloidal material in soil indicated by heat of wetting ratio	Colloidal material in soil indicated by adsorption ratios		
		Dye	H <sub>2</sub> O	NH <sub>3</sub>
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Cecil clay loam subsoil.....	35.6	25.4	31.5	26.0
Chester loam soil.....	15.3	11.0	8.5	16.7
Marshall silt loam soil.....	30.3	26.3	27.3	33.6
Miami silty clay loam subsoil.....	54.2	54.8	50.1	54.2
Norfolk fine sandy loam subsoil.....	25.0	23.7	20.5	18.6
Sassafras silt loam subsoil.....	22.5	13.2	18.9	21.8
Sharkey clay soil.....	58.8	56.6	61.2	58.8
Wabash silt loam soil.....	29.5	29.4	29.8	34.5

The colloidal contents of the various soils indicated by the heat of wetting ratio agree well with the contents shown by one or more of the adsorption ratios. The heat of wetting ratio of soil and colloid obviously affords a more accurate estimation of the colloid content of a soil than the heat of wetting of the soil alone. It is probable, however, that even when heats of both soil and colloid are obtained the determination of the quantity of colloid in a soil is in most cases not strictly accurate, inasmuch as the heat of wetting ratios may be subject to the same inaccuracies as were found to affect adsorption ratios. A previous investigation (4) indicated that in estimating the colloidal content of a soil from the adsorption ratio of soil and colloid there might be two sources of inaccuracy, the possibility of the sample of colloid extracted not being representa-

tive in adsorptive capacity of all the colloidal material in the soil; and the possibility of the colloidal material having a different adsorptive capacity after isolation from what it had in the unextracted soil.

While it is a relatively simple process to isolate a small portion of the colloidal material from a soil, it is a long and tedious operation in most cases to extract as much as 50 per cent of the total colloid present. The data given in Tables I and II were obtained using samples which comprised not more than 5 per cent of the total colloidal material in the soil. Previous work indicated that such small samples differed by only about 10 per cent in adsorptive capacity from the bulk of the colloid that it was possible to extract. This small variation was shown by extracting successive portions of the colloidal material from several soils and testing the adsorptive capacities of the different fractions. A few of these samples which were not exhausted in the previous investigation were tested for heat of wetting. The results are shown in Table III, together with the adsorption data previously determined (4, p. 18).

TABLE III.—*Heats of wetting of colloid samples successively extracted from the same soil*

	Heat of wetting per gram of sample	Malachite green adsorbed per gram of sample	Water adsorbed per gram of sample	Ammonia adsorbed per gram of sample
	<i>Calories</i>	<i>Gram</i>	<i>Gram</i>	<i>Gram</i>
Vega Baja clay loam soil:				
First fraction of colloid.....	4.8	0.0584	0.3125	0.0206
Second fraction of colloid.....	4.4	.0463	.2749	.0165
Marshall silt loam soil:				
First fraction of colloid.....	12.2	.3159	.3090	.0611
Fourth fraction of colloid.....	9.8	.1916	.2328	.553

The variation in the heats of wetting of successively extracted portions of colloid was very similar to the variations in adsorptive capacities of the same samples. It is, therefore, to be assumed that the conclusion reached regarding adsorptive capacities on the basis of more extensive data applies in a general way to heat of wetting. That is to say, a small sample of colloid has, as a rule, only a slightly different heat of wetting from that of a sample which is a larger part of all the colloid.

Although the extractable part of the colloidal material of a soil may be fairly uniform in character, it does not follow that the part which is exceedingly difficult to isolate necessarily has the same properties. In determining the representativeness of a colloid sample with respect to heat of wetting it is important to consider also that portion which is not extractable by the ordinary means. In several instances separation of colloidal material has been made as completely as present methods will permit. By long and painstaking work of extraction 50 to 90 per cent of the total colloidal material present has been isolated from certain soils. The residues left after these extractions contained the so-called "unextractable" colloid. The heats of wetting of these residues were obtained; the percentages of colloid present were determined by W. H. Fry microscopically by a method previously described (3); and from these data the heats of wetting of the unextracted colloids were calculated.

Table IV gives a comparison of the heat of wetting of the extracted and of the unextracted colloidal material, together with data from which the heat of wetting of the unextracted colloid was calculated.

TABLE IV.—*Heats wetting of extracted and of unextracted colloidal material in soils*

Soil type	Determined heat of wetting per gram of colloid extracted from soil	Heat of wetting per gram of residue after extracting colloid	Colloid in residue by microscopical determination	Calculated heat of wetting per gram of colloid left in residue
	<i>Calories</i>	<i>Calories</i>	<i>Per cent</i>	<i>Calories</i>
Houston clay soil.....	11.6	2.1	40	5.2
Houston clay subsoil.....	10.5	1.2	32	3.8
Durham loam subsoil.....	5.3	1.9	11	17.3
Vega Baja clay soil.....	4.6	4.8	97	5.0

<sup>a</sup> This fine fraction contains considerable amounts of mica which may be responsible for a significant amount of this heat value.

The results given in Table IV indicate in three of the four cases studied a wide difference in the heat values of the extracted and unextracted colloids. These results are in accord with the data obtained in adsorption studies. It thus appears that in some soils, the part of the colloidal material which is difficult to isolate may have a very different heat of wetting from the part which is readily extracted. This fact tends to bring about more or less inaccuracy in estimating the colloidal content of a soil from the ratio of heats of wetting of soil and sample of colloid.

Evidence has been obtained that in the process of isolating and concentrating the colloidal material the adsorptive capacity of the colloid may be more or less altered. Apparently the heat of wetting property may also be subject to alteration.

Since practically all of the heat of wetting of a soil is due to the colloidal material, the ratio,

$$\frac{\text{Heat of wetting of soil}}{\text{Heat of wetting of colloid}},$$

should show the true quantity of colloid in the soil when the ratio is based on a fair sample of all the colloidal material present, unless the colloidal material after extraction has a different heat of wetting capacity from what it has in the untreated soil. By very thorough work, I. A. Denison, of this bureau, has isolated 76 to 88 per cent of the total colloidal material present in three soils. Owing to the completeness of the extraction, the colloidal material must be very nearly representative of all the colloid in the soils. Nevertheless, Table V shows that when heats of wetting of these samples are used in the ratio, quantities of colloidal material are indicated which are considerably in excess of true values. The true quantity of colloid in the soil is taken as the quantity actually isolated plus the quantity of unextracted colloid left in the residues estimated microscopically.

Table V gives the quantity of colloid in the soil indicated by the ratio and the heat of wetting values from which the ratio is calculated. The quantities of colloid extracted and true values for colloid content of the soil are also given in this Table.

The differences between the quantities of colloid indicated by heat of wetting ratios and by the combined extraction and microscopical determinations can not reasonably be attributed to failure to obtain a representative sample of the colloidal material for the heat of wetting ratios. Practically all the colloidal material was extracted for a sample. Moreover, it has already been shown that the so-called "unextractable" colloid usually has a lower heat of wetting capacity than the extracted material. Hence, if all the colloid had been extracted, to produce perfect samples, the heat of wetting ratios would presumably have been

slightly higher than the values obtained which were already too high. It would seem that the high values of the heat of wetting ratios are due to a reduction in the heat of wetting capacity of the colloid produced by extraction.

TABLE V.—*True colloid content of soil compared with content indicated by heat of wetting ratio when ratio is based on a large sample of colloid obtained by almost complete extraction of the soil*

Soil type	Heat evolved per gram of soil	Heat evolved per gram of colloid extracted	Colloid content as calculated by heat of wetting ratio	Colloid extracted as per cent of whole soil	Colloid left in residues as per cent of whole soil	True colloid content of soil
	<i>Calories</i>	<i>Calories</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Houston clay soil.....	9.5	11.6	89.1	53	17	70
Houston clay subsoil.....	10.8	10.5	102.9	66	10	76
Durham loam subsoil.....	2.2	5.3	41.5	16	2	18

More conclusive evidence that the process of extraction alters the heat of wetting of the colloid is obtainable from some of the data given in Tables IV and V. It can be shown that the sum of the heats of wetting of the extracted and unextracted colloids in the soil is in some cases appreciably less than the heat of wetting of the untreated soil. This would seem to show quite conclusively that in the cases studied the colloidal material as it exists in the untreated soil has a higher heat of wetting capacity than the colloid as it exists after isolation.

Table VI compares the heat of wetting of the untreated soil with the heats of wetting of the combined fractions into which the soil is separated by the process of extraction. The data for this comparison are given in Tables IV and V.<sup>7</sup>

TABLE VI.—*Influence of extraction upon the heat of wetting of soil colloids*

Soil type	Heat evolved by 1 gm. of untreated soil	Heat evolved by the extracted and unextracted colloid contained in 1 gm. of soil	Loss in heat of wetting per gram of soil due to process of extraction	Loss expressed as per cent of heat evolved by untreated soil
	<i>Calories</i>	<i>Calories</i>	<i>Calories</i>	<i>Per cent</i>
Houston clay loam soil.....	9.5	7.5	2.0	21
Houston clay loam subsoil.....	10.8	7.3	3.5	32
Durham loam subsoil.....	2.2	1.2	1.0	46
Vega Baja clay loam <sup>a</sup> .....	4.0	3.5	.5	13

<sup>a</sup> The colloid of the Vega Baja soil was not so completely extracted as in the case of the other three soils given in this Table. Details regarding the extraction of this sample are given in a previous publication of this bureau (4). The extracted colloid amounted to 29.3 per cent of the soil while the soil contained a total of 72.4 per cent of colloid.

The process of extraction in each case reduced the heat of wetting values of the soil material. In two cases the reduction in heat of wetting was of about the same order as the probable error. In the other two cases, however, the differences were sufficiently great to be of undoubted significance. These results are

<sup>7</sup> For example, the heat of wetting of the Houston soil is 9.5 calories per gram and the colloid extracted from it 11.6 calories per gram (Table V). This soil contains 53 per cent extractable colloid (Table V). The colloid extracted from 1 gm. of soil would, therefore, give 6.6 calories. This soil also contains 17 per cent of unextracted colloid (Table V) which gives a heat value of 5.3 calories per gram (Table IV). The unextracted colloid in 1 gm. of soil, therefore, amounts to 0.9 calories, making a total of 7.5 calories for the fractions from 1 gm. of soil after extraction, as compared with 9.5 calories for the untreated soil.

in accord with evidence of alteration which was obtained in the case of adsorption studies where water vapor and ammonia were the substances adsorbed (4).

It is not clear just how the process of extraction influences the heat of wetting of the colloid. It may be influenced to some extent at least by the structure of the aggregates of the colloidal material. The state of aggregation or structure of the particles in the colloidal material probably has considerable effect upon its heat of wetting and other physical properties. The same colloidal material will doubtless vary in its active surface according to whether the primary particles are present in loose or in compact aggregations. The arrangement of the colloidal particles is no doubt changed through the process of dispersion, subsequent concentration and drying. It is reasonable to assume that such changes as these are largely responsible for the alteration in heat of wetting values and may be responsible for the alteration in adsorptive capacity for ammonia or water.

In estimating the colloid content of a soil from the ratio,

$$\frac{\text{heat of wetting of soil}}{\text{heat of wetting of colloid}},$$

the value should be too high, provided alteration were the only source of inaccuracy. It has been shown, however, that the extracted colloids usually give higher heat of wetting values than the so-called "unextractable" portions. Furthermore, a small portion of the colloid extracted from a soil tends to give a higher heat of wetting than a larger sample (Table III). The error due to the quantity of colloid sample extracted and the error due to alteration, are, therefore, in opposite directions. If both errors were of the same magnitude the heat of wetting ratio should give a true expression of the quantity of colloid in a soil. Inasmuch as the sampling error tends to be less the larger the sample and the alteration error is probably greater the larger the sample, it is presumable that some size of sample from each particular soil would give a correct value for colloid content by the heat of wetting ratio. It is apparent that in case of very large samples (Table V) the alteration error was more than sufficient to balance the sampling error.

Ammonia adsorption studies indicate an alteration in adsorbing power due to extraction and also a sampling error similar to that found for heat of wetting (4). The colloid content of soils shown by heat of wetting ratios and by ammonia ratios (Table II) are as a rule in good agreement. Although complete confirmation is lacking, it seems probable that the small samples of colloid used in these ratios give errors so nearly compensating that the results usually approximate the true quantities of colloid present in the soils.

The heat of wetting method and the ammonia adsorption method for determining the colloidal content of soils appear to be of about the same value as regards practicability. Both require the extraction of a sample of the colloidal material, which involves considerable time as well as expense for apparatus. They do not appear to be as practicable as, and there is no evidence of their being more accurate than, the water adsorption method when the adsorption is conducted over 3 per cent sulphuric acid and a factor of 0.3 gm. of water per gram of colloid is taken as the constant value for soil colloids.

### SUMMARY

The heats of wetting of soils and of colloidal material isolated from soils are given and the possibility of determining the colloidal content of soils from such data is discussed.

Heats of wetting of the colloidal materials from different soils vary widely. In some cases they are comparable with the heat values for starch and for synthetic inorganic gels.

Practically all of the heat of wetting of a soil appears to be due to colloidal material.

Although the colloid content of a soil can not be deduced from the heat of wetting of the soil alone, a fair approximation of colloid content may be indicated by the formula,

$$\frac{\text{heat of wetting of soil}}{\text{heat of wetting of colloid}} \times 100.$$

This method is subject to some inaccuracy, due to failure to isolate a sample representative of the whole of the colloid, and due to alteration of the colloid through the process of extraction. These errors, however, may be in opposite directions and in certain cases compensating. On the whole, the heats of wetting of soil and colloid probably indicate the colloid content of a soil about as accurately as adsorption determinations.

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# COMPARISON OF PIMA COTTON WITH UPLAND VARIETIES IN ARIZONA<sup>1</sup>

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## INTRODUCTION

From the time the cotton industry in Arizona reached commercial importance, about 1915, until the season of 1922, the Salt River Valley of Arizona was maintained as a one-variety cotton community and received wide recognition as a source of production for American Egyptian cotton of high quality. Marketing conditions have not been favorable for Egyptian cotton since the war period, and each year larger quantities of Upland cotton have been grown. Only a few farmers planted Upland cotton in 1921, but in 1922 there were about 4,300 acres, and in 1923 about 58,000 acres. On the other hand, the planting of Pima cotton has been reduced from 73,000 acres in 1922 to about 40,000 acres in 1923. The future planting policy in the Salt River Valley is likely to be determined largely with reference to market conditions and prices.

Plantings have been made in each season since 1920 at the United States Field Station at Sacaton, Ariz., to compare the habits of growth and productiveness of the varieties considered best adapted for this region. The experiments consisted of quarter-acre blocks of seven rows of each variety, and the rows picked and recorded separately. The yield relations of the varieties differed with the seasons, but the plantings were not repeated in the way that is necessary to determine whether such differences are significant. Hence the results are not reported in an attempt to prove the superiority of any variety.

## VARIETIES STUDIED AND METHODS EMPLOYED

In 1920 two Upland varieties, Lone Star and Durango, were compared with the Pima Egyptian variety. In 1921 the Acala variety was added to the series, and in 1923 the Hartsville and Mebane. The Acala is an Upland variety of Mexican origin which in recent years has been grown extensively in the San Joaquin and Coachella Valleys of California, in Oklahoma and northern Texas, and is rapidly increasing in popularity in Arizona and in the southwestern cotton growing States. The Hartsville is a long staple Upland variety from South Carolina originated by the United States Department of Agriculture and first distributed in 1907. The Mebane is a well known Texas big-boll variety with  $1\frac{1}{8}$ -inch staple. All of these varieties were planted to some extent in the Salt River Valley during 1922 and 1923, and are the only varieties that have received attention from the growers except a so-called "Delta" cotton which is indistinguishable from Hartsville.

The soil at Sacaton is an alluvial deposit of fine sand and silt, technically known as Pima sand, which has a high degree of fertility and a large water-holding capacity, making it very well adapted to cotton growing. The field used for the experiments is one of the most uniform on the station and offers special advantages for experimental studies of cotton.

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<sup>1</sup> Received for publication Mar. 28, 1924—issued Nov., 1924.



The experiments were planted in adjacent blocks of seven rows of each variety, the same field being used each year, though the varieties were not kept in the same order. By referring to Table X the plats on which a particular variety was planted in each year can be ascertained.

The plantings were made as soon as danger of frost was past with a two-row planter adjusted for rows 44 inches apart. Seeding was at the rate of about 35 pounds of seed per acre. This is a somewhat higher rate than is generally used by commercial growers, but it has been found that this amount is required to insure a uniform stand which is necessary in experimental plantings. The date when the varieties were planted each year is given in Table I.

The height of the seedling plants when thinned varied somewhat in different years, but usually they averaged about 12 inches high, and were in no case allowed to grow over 15 inches in height before thinning. In all four years the plants were thinned as nearly as possible to one foot apart in the rows, which is the spacing most used by Pima cotton growers in Arizona. Thinning was never completed earlier than May 28 nor later than June 13.

TABLE I.—*Dates of planting variety tests at Sacaton, Ariz.*

Variety	1920	1921	1922	1923
Pima.....	Apr. 2	Apr. 9	Mar. 27	Apr. 18
Lone Star.....	do.	do.	<sup>a</sup> May 10	Do.
Durango.....	do.	do.	Mar. 27	Do.
Acala.....		do.	Apr. 8	Do.
Mebane.....				Do.
Hartsville.....				Do.

<sup>a</sup> In 1922 Lone Star was first planted on Mar. 27 but poor germination and cold weather killed many of the young plants and further affected the stand, which necessitated the entire replanting of this plat on May 10.

Indications of water stress in Pima plants are easily recognized, and the irrigation problem is not very difficult after one becomes familiar with the characteristics of the plants. In the early stages of development, the irrigation water was applied at such intervals as was necessary to keep the plants from wilting, except during short periods in the middle of the day, and to prevent the upper fruiting branches from projecting above the terminal buds of the central stalk, which is an indication that growth is being checked. After flowering commenced the time of irrigation was governed largely by the position of the flowers. An effort was made to keep the flowers from appearing at the tops of the plants, except during the fall months when upward growth of the plants had practically ceased.

Plant behavior which indicates the proper time for irrigating the Upland varieties is much more difficult to recognize than with Pima. The flowers are hidden more by the leaves and do not appear at the tops of the plants until the cotton has been in distress for several days from lack of water. General wilting of Upland plants at any time of day during the summer months is usually an indication that water has been withheld too long.

The dates when water was applied to the different varieties in 1922 and 1923 are shown in Table II. Days on which more than 1 inch of rain fell are also included, as such rains were considered as the equivalent of an irrigation. These data are given for 1922 and 1923 only, as no detailed notes were taken in the previous years on flowering, shedding, and plant growth, in connection with which irrigation dates might be of interest.

TABLE II.—*Dates when variety plantings were irrigated and when over 1 inch of rainfall occurred at the United States Field Station, Sacaton, Ariz., in 1922 and 1923<sup>a</sup>*

1922

Month	Date of rain	Date of irrigation			
		Variety			
		Acala	Durango	Pima	Lone Star
April.....	-----	-----	28	28	-----
July.....	-----	7	7	7	7
Do.....	-----	25	25	25	-----
August.....	-----	-----	-----	-----	1
Do.....	-----	-----	-----	8	8
Do.....	15	25	-----	-----	-----
Do.....	-----	-----	-----	-----	-----
September.....	31	-----	-----	-----	-----
Do.....	-----	-----	-----	• 15	-----
Do.....	-----	19	19	-----	-----

1923

Month	Date of rain	Date of irrigation					
		Variety					
		Harts-ville	Durango	Pima	Acala	Mebane	Lone Star
May.....	-----	29	29	29	29	29	29
June.....	-----	29	29	29	29	29	29
July.....	13	-----	-----	-----	-----	-----	-----
Do.....	-----	24	24	24	24	24	24
August.....	-----	-----	-----	-----	-----	6	6
Do.....	-----	7	7	7	7	-----	-----
Do.....	-----	21	21	21	21	21	21
September.....	-----	1	1	1	1	1	1
Do.....	-----	11	11	11	11	11	11

<sup>a</sup> On Aug. 25, 1922, and on Oct. 2, 1923, the varieties were all watered to permit the proper maturing of the crop and had no effect on the shedding.

In 1922 and 1923 after the plants were thinned, and before any flowers had opened, a section consisting of 25 representative plants was selected in one of the interior rows of each variety. Detailed notes were taken daily on the behavior of these plants throughout the remainder of the season.

The height of the individual plants in each of the 25-plant sections was measured at weekly intervals through the season until growth had practically ceased. The number of flowers produced each day was recorded from the time of the appearance of the first flower until September 15. A small white tag bearing the date of flowering was fastened to the pedicel of each flower in such a manner that it remained attached to the young boll, even after shedding. Collections were made daily of the tags which had fallen with shed bolls, and the dates recorded. Thus a record of flowering and shedding was obtained for each day throughout the season. In 1923 the date of opening was recorded for each boll, which determined the period required for each boll to reach maturity.

# VARIETAL DIFFERENCES IN PLANT GROWTH AND LENGTH OF ACTIVE GROWING PERIOD

In 1922 and 1923 measurements of height of the individual plants in the 25-plant sections of each variety were begun in the last week of June and continued for 12 weeks, or through the second week of September, covering the same period in which the flowering and shedding notes were taken. The average height of the plants of each variety at weekly intervals, and the increase in height over the previous week, are shown in Table III for 1922 and Table IV for 1923.

TABLE III.—Average height in inches of 25 plants of each variety for each week, and the increase over the previous week, throughout the fruiting season of 1922

Date	Acala		Durango		Pima		Lone Star	
	Average height	Average increase	Average height	Average increase	Average height	Average increase	Average height	Average increase
June 26.....	22.4		18.2					
July 3.....	27.1	4.7	23.1	4.9	24.8		19.9	
10.....	30.2	3.1	25.9	2.8	28.9	4.1	23.8	3.9
17.....	35.2	5.0	31.8	5.9	32.6	3.7	28.4	4.6
24.....	40.4	5.2	35.9	4.1	35.9	3.3	30.7	2.3
31.....	45.9	5.5	40.4	4.5	40.3	4.4	31.2	.5
Aug. 7.....	51.6	5.7	43.3	2.9	42.3	2.0	31.3	.1
14.....	54.2	2.6	43.8	.5	43.9	1.6	31.4	.1
21.....	55.2	1.0	44.3	.5	47.1	3.2	31.4	0
28.....	56.0	.8	44.6	.3	48.0	.9	31.4	0
Sept. 4.....	56.0	0	44.6	0	48.0	0	31.4	0
11.....	56.0	0	44.6	0	48.0	0	31.4	0

TABLE IV.—Average height in inches of 25 plants of each variety for each week, and the increase over the previous week, throughout the fruiting season of 1923

Date.	Hartsville		Durango		Pima		Acala		Mebane		Lone Star	
	Average height	Average increase	Average height	Average increase	Average height	Average increase	Average height	Average increase	Average height	Average increase	Average height	Average increase
June 25.....	17.7		20.0		19.3		19.2		18.5		15.8	
July 2.....	23.5	5.8	24.9	4.9	24.4	5.1	25.0	5.8	23.5	5.0	20.2	4.4
9.....	29.6	6.1	30.6	5.7	31.0	6.6	30.6	5.6	28.4	4.9	24.9	4.7
16.....	33.6	4.0	36.0	5.4	36.4	5.4	34.7	4.1	31.3	2.9	27.8	2.9
23.....	36.0	2.4	40.1	4.1	41.4	5.0	36.6	1.9	32.4	1.1	29.3	.5
30.....	37.0	1.0	42.1	2.0	46.4	5.0	37.8	1.2	33.2	.8	29.6	1.3
Aug. 6.....	37.3	.3	42.7	.6	50.4	4.0	38.3	.5	33.4	.2	29.8	.2
13.....	37.6	.6	42.9	.2	54.5	4.1	38.5	.2	33.4	0	29.8	0
20.....	37.8	.2	42.9	0	57.0	2.5	39.0	.5	33.4	0	29.8	0
27.....	38.0	.2	42.9	0	58.2	1.2	39.3	.3	33.4	0	29.8	0
Sept. 3.....	38.0	0	42.9	0	58.4	.2	39.4	.1	33.4	0	29.8	0
10.....	38.0	0	42.9	0	58.4	0	39.6	.2	33.4	0	29.8	0

It may be seen that in both these years the Durango, Acala, and Pima plants had a much longer period of active growth than Lone Star. The comparatively short growing period of Lone Star was also noticed in 1920 and 1921. The plant growth of Mebane in 1923 was much like Lone Star, although Mebane became slightly taller. These two varieties were the only ones which showed a decidedly shorter growth period than Pima, although the growing period of Durango, in both 1922 and 1923, was intermediate between these two varieties and Pima.

In 1923 the Pima plants continued in active growth for nearly a month after all the Upland varieties had practically ceased growing, but in 1922 the Acala was planted 12 days later than Pima and the plants continued to grow at nearly the same rate as Pima to the end of the season. Acala and Durango in 1923 ceased rapid growth much earlier than in the previous year, while the growth behavior of Pima and Lone Star in 1923 closely resembled their previous performance in 1922.

Figures 1 and 2 show in graphic form the growth rate data contained in Tables III and IV, which makes a direct comparison of the height and length of the growing period of the different varieties easier.

### DIFFERENCES IN FLOWER PRODUCTION

The number of flowers produced each day in the 25-plant sections, the number of bolls that were shed, and the number of bolls that were retained and developed in the seasons of 1922 and 1923, are given in Tables V and VI. The Upland varieties began to flower about a week before Pima with the exception of the replanted Lone Star in 1922. Also the Upland varieties usually produce a larger number of flowers than does Pima, but this advantage is reduced by much greater shedding after flowering among the Upland varieties, so that

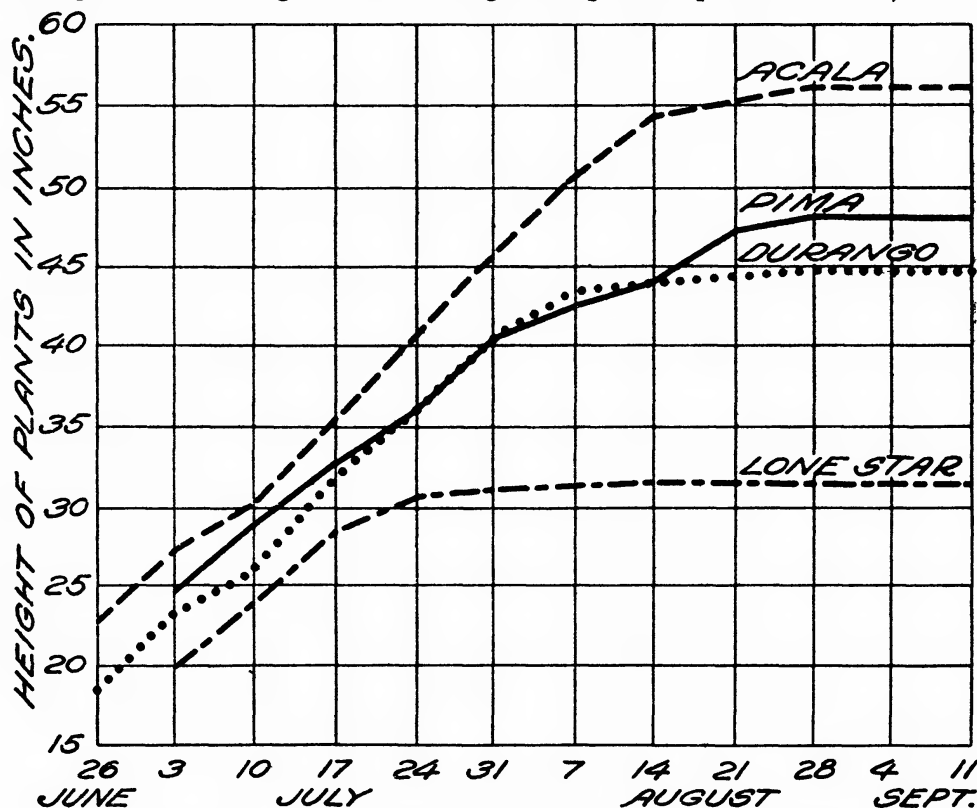


FIG. 1.—Average rate of growth for 25 plants in each variety from June 26 to September 11, 1922.

Pima matures more bolls than any of the Upland varieties. On the other hand, the greater number of Pima bolls is counterbalanced by the larger size of the Upland bolls.

It will be seen from figure 3 that the relations of the varieties in flower production were much the same in 1923 as in the previous year. The Acala variety produced on 25 plants a total of 1,948 flowers during the active flowering period of 1922, with a daily average of 23.4, while in 1923 only 1,290 flowers were produced, and the daily average was only 15.9. Such a difference may be attributed to soil variations which were not distinguishable when the 25 plants were selected for record purposes. In 1922 many of the late bolls on some of the varieties failed to reach maturity on account of an early frost, whereas in 1923 the season was longer, and practically all of the bolls matured and were picked. This accounts, in part, for the lack of a correlation between the flower production and yields in 1922 and 1923.

In 1922 the Durango plants continued flowering for 86 days, which was the longest period of active flowering recorded in either 1922 or 1923. In 1923, however, the active production of flowers by the Durango plants continued for

only 61 days, while Acala produced flowers for 81 days. The short flowering season of Lone Star in both years is noticeable in figure 3. Nevertheless, the total number of flowers and also the yield of this variety do not compare unfavorably with the varieties which continued to flower much longer. Mebane also had a very short flowering period in 1923 but was able to set a large number of bolls.

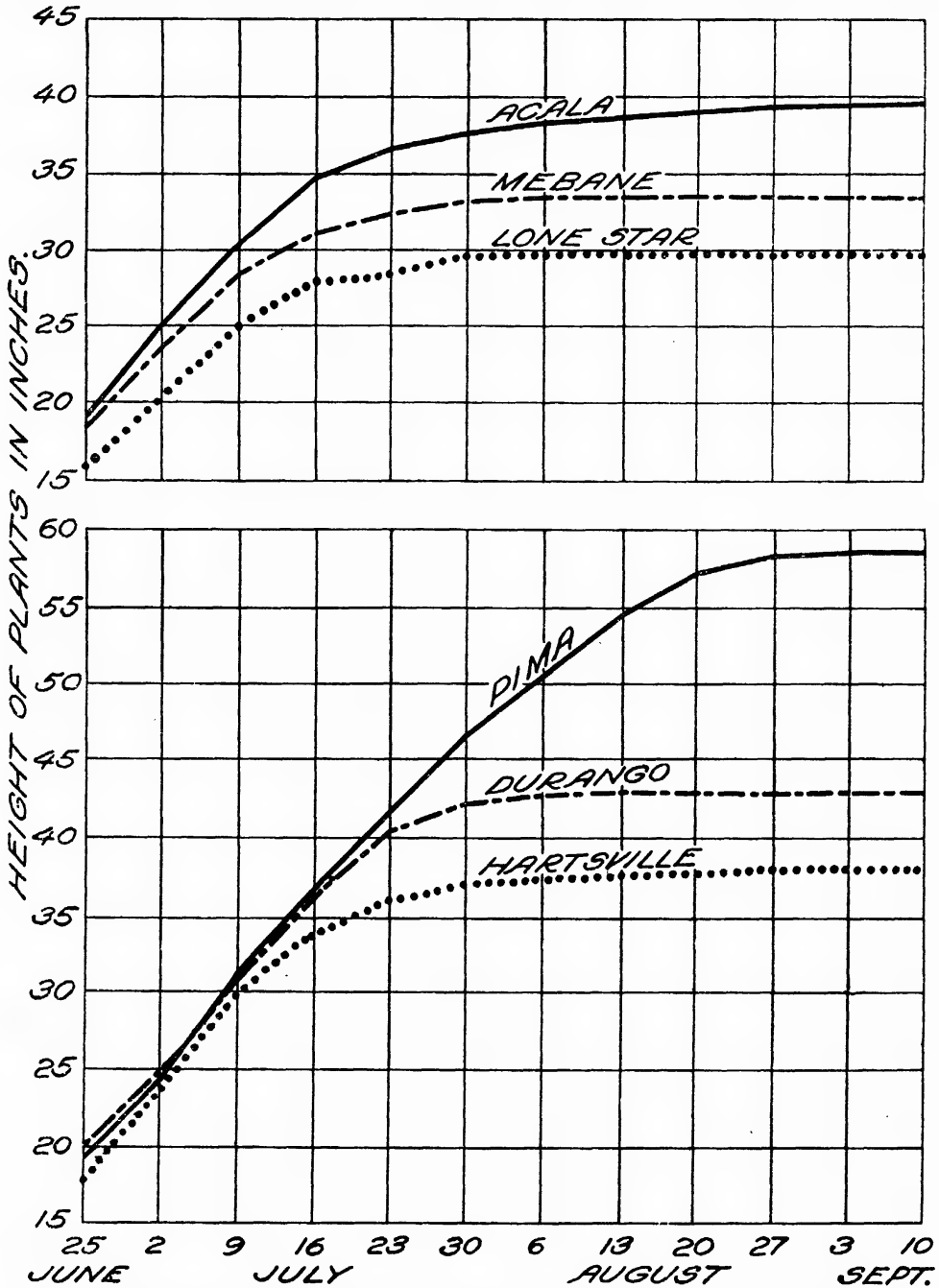


FIG. 2.—Average rate of growth for 25 plants in each variety from June 25 to September 10, 1923.

The period of active flowering is apparently closely related to the period of active growth of the plants, which is shown by comparing figure 3 with figures 1 and 2. Under the conditions of the experiment the short growing season of Lone Star was also reflected in the short flowering season, and the lengths of the flowering periods of the other varieties are also proportionate to the length of their respective growing seasons, except that flowering may cease through shedding of squares. The Pima plants, however, though they continued growing

later in the fall than the other varieties, had a shorter period of active flowering than Acala or Durango in 1922, and shorter than Acala or Hartsville in 1923. Occasional flowers appeared in all of the varieties after the regular flower counts were discontinued. It was noted that this casual flowering continued longer in Pima than in other varieties.

#### COMPARISON OF BOLL SHEDDING

Egyptian and Upland cottons differ considerably in susceptibility to shedding, both of squares and of young bolls. Shedding has been studied in Upland cotton by Lloyd (4, 5)<sup>2</sup>, Ewing (2) and others in the United States, and in Egyptian cotton by Balls (1). Lloyd found in Alabama that about 50 per cent of the shedding in Upland varieties consisted of squares, but Ewing asserts that shedding in the square stage is relatively unimportant in Mississippi. Balls reports that "shedding in Egypt takes place almost entirely in the flower stage," probably

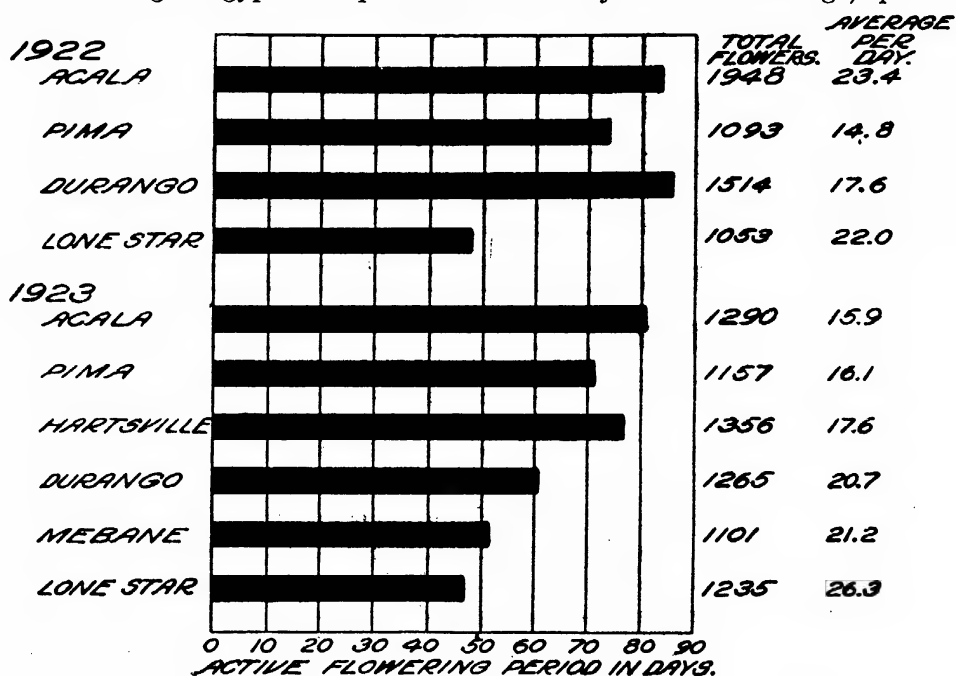


FIG. 3.—Active flowering period in days.

meaning 2 or 3 days after flowering. In Arizona it has been found that most of the shedding in both Egyptian and Upland varieties occurs soon after flowering, before the bolls are 10 days old. Under some conditions, however, the shedding of squares also becomes an important factor and causes reduction in yields.

Shedding data covering two seasons at Sacaton, Ariz., are contained in Tables V and VI. Pima cotton in 1922 retained 82.5 per cent of its bolls and in 1923 70.7 per cent, under conditions where Upland varieties usually retained less than 30 per cent of their bolls.

The greater ability to retain its squares and bolls enables the Pima Egyptian cotton to produce satisfactory yields in the Southwestern States, notwithstanding the smaller bolls. In some years the shedding of Pima bolls at Sacaton has been even less than in this experiment. In 1920 Martin and Loomis (7) reported a loss of only 11.2 per cent from a total of 10,317 flowers. Willett<sup>3</sup> recorded a loss of 15.8 per cent in 1922 from a total of 7,291 flowers. Other data on boll shedding in 1923 showed Pima at Sacaton retaining 86.4 per cent of the bolls, and Acala 30.5 per cent, while at Shafter, Calif., 90.2 per cent of Pima bolls were retained, and only 31 per cent of Acala bolls.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 953.

<sup>3</sup> Unpublished data communicated by Dr. T. H. Kearney. The data from Sacaton in 1923 were secured by Max Willett and Dow D. Porter, and those from Shafter by R. H. Peebles.

The data in Table VI show that the greatest amount of shedding of all varieties for both 1922 and 1923 occurred during the period from July 10 to August 20, when temperatures were highest and abrupt weather changes occurred.

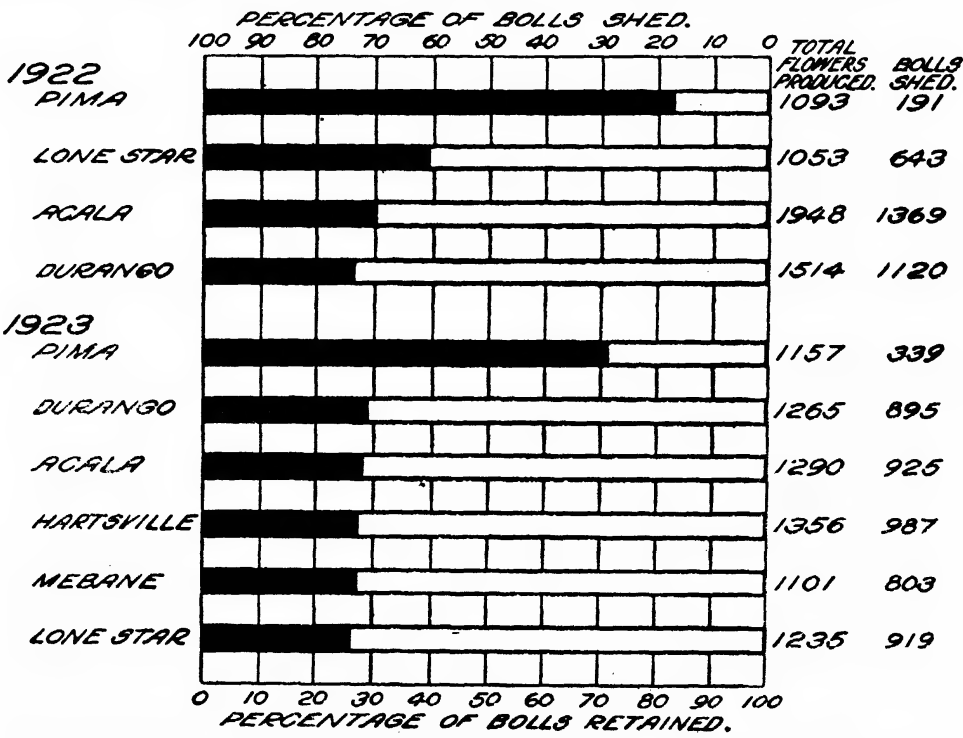


FIG. 4.—Showing percentage of bolls shed and bolls retained on 25 representative plants of each variety in 1922 and 1923

In figure 4 the percentage of bolls shed and bolls matured by each variety for 1922 and 1923 is shown graphically. The large proportion of bolls matured and the correspondingly small number of bolls shed, conspicuously characterize the Pima variety in comparison with the Upland varieties.

TABLE V.—Number of flowers produced, and bolls shed per day, and bolls matured by 25 representative plants of 4 cotton varieties in 1922

Date	Variety											
	Acala			Durango			Pima			Lone Star		
	Number flower- ers	Bolls shed	Ma- ture bolls	Number flower- ers	Bolls shed	Ma- ture bolls	Number flower- ers	Bolls shed	Ma- ture bolls	Number flower- ers	Bolls shed	Ma- ture bolls
June 22				3	3	0						
23	1	0	1	1	1	0						
24	1	1	0	1	1	0						
25	1	1	0	1	0	1						
26	1	0	1	2	1	1						
27	5	2	3	5	2	3						
28	4	1	3	2	2	0						
29	1	1	0	3	1	2	2	0	2			
30	8	4	4				2	0	2			
July 1	5	0	5	4	1	3	2	1	1			
2	6	3	3	2	2	0	1	0	1			
3	6	6	0	4	4	0	3	0	3			
4	10	4	6	3	2	1	4	1	3			
5	12	7	5	3	3	0	4	3	1			
6	22	18	4	13	11	2	6	0	6	2	1	1
7	20	12	8	13	9	4	2	0	2	3	3	0
8	19	10	9	7	4	3	8	1	7	4	3	1
9	24	17	7	15	12	3	11	0	11	2	1	1

TABLE V.—*Number of flowers produced and bolls shed per day, and bolls matured by 25 representative plants of 4 cotton varieties in 1922—Continued*

Date	Variety											
	Acala			Durango			Pima			Lone Star		
	Number flow- ers	Bolls shed	Ma- ture bolls	Number flow- ers	Bolls shed	Ma- ture bolls	Number flow- ers	Bolls shed	Ma- ture bolls	Number flow- ers	Bolls shed	Ma- ture bolls
July 10.....	24	18	6	13	9	4	8	1	7	1	1	0
11.....	15	11	4	8	8	0	9	1	8	1	1	0
12.....	31	24	7	11	6	5	3	1	2	4	4	0
13.....	26	22	4	9	8	1	5	1	4	2	2	0
14.....	21	18	3	8	7	1	8	1	7			
15.....	16	12	4	5	2	3	7	0	7	5	3	2
16.....	30	19	11	28	14	14	7	0	7	17	2	15
17.....	24	16	8	10	8	2	6	1	5	14	1	13
18.....	29	23	6	17	7	10	8	0	8	20	8	12
19.....	24	17	7	12	7	5	3	0	3	24	8	16
20.....	11	10	1	10	6	4	8	2	6	16	5	11
21.....	31	23	8	24	13	11	10	3	7	43	17	26
22.....	37	28	9	15	10	5	12	3	9	29	18	11
23.....	28	22	6	11	8	3	7	1	6	18	13	6
24.....	15	14	1	8	8	0	10	2	8	33	21	12
25.....	35	29	6	21	13	8	16	6	16	51	44	7
26.....	34	25	9	22	11	11	14	0	14	42	32	10
27.....	32	27	5	33	19	14	21	2	19	65	50	15
28.....	38	24	14	35	27	8	16	0	16	53	28	25
29.....	28	21	7	26	20	6	10	0	10	50	28	22
30.....	24	14	10	31	25	6	18	1	17	46	14	32
31.....	28	19	9	35	22	13	12	1	11	54	24	30
Aug. 1.....	46	26	20	48	35	13	14	0	14	72	43	29
2.....	37	25	12	25	23	2	21	2	19	49	35	14
3.....	27	14	13	43	30	13	16	6	10	43	29	14
4.....	35	21	14	46	35	11	25	7	18	49	36	13
5.....	49	37	12	52	40	12	32	21	11	49	39	10
6.....	43	28	15	59	52	7	24	15	9	52	35	17
7.....	40	27	13	41	33	8	17	2	15	24	18	6
8.....	50	38	12	57	45	12	36	1	35	27	18	9
9.....	48	29	19	56	48	8	28	1	27	30	18	12
10.....	41	27	14	61	54	7	37	2	35	16	10	6
11.....	37	27	10	39	32	7	22	2	20	8	4	4
12.....	35	22	13	32	30	2	36	2	34	7	6	1
13.....	34	27	7	59	45	14	41	4	37	15	10	5
14.....	51	41	10	41	24	17	28	0	28	5	3	2
15.....	58	36	22	43	25	18	40	2	38	4	4	0
16.....	38	29	9	55	38	17	32	4	28	2	1	0
17.....	46	37	9	38	31	7	32	4	28	1	1	0
18.....	32	21	11	19	17	2	47	10	37			
19.....	34	28	6	27	25	2	24	7	17			
20.....	36	27	9	22	19	3	29	9	20			
21.....	33	23	10	16	16	0	40	11	29			
22.....	24	14	10	21	15	6	25	6	19	1	1	0
23.....	33	31	2	16	15	1	26	9	17			
24.....	36	31	5	13	12	1	13	7	6			
25.....	34	28	6	12	12	0	11	7	4			
26.....	19	18	1	8	7	1	3	3	0			
27.....	26	22	4	13	9	4	2	2	0			
28.....	31	29	2	9	7	2	3	2	1			
29.....	27	19	8	14	7	7						
30.....	17	14	3	18	4	14						
31.....	23	12	11	6	3	3						
Sept. 1.....	21	7	14	7	3	4						
2.....	10	5	5	3	1	2	5	1	4			
3.....	8	4	4	1	1	0	16	1	15			
4.....	16	8	8	2	1	1	13	3	10			
5.....	5	2	3				6	1	5			
6.....	8	3	5	2	0	2	7	1	6			
7.....	6	3	3	3	3	0	26	0	26			
8.....	7	3	4	4	3	1	8	0	8			
9.....	4	1	3	1	1	0	8	1	7			
10.....	5	1	4				5	0	5			
11.....	3	0	3				11	1	10			
12.....	4	1	3	3	1	2	11	1	10			
13.....	3	0	3	4	1	3	7	0	7			
14.....	1	0	1				3	0	3			
15.....				1	0	1						
Total.....	1,948	1,369	579	1,514	1,120	394	1,093	191	902	1,053	643	410
Per cent shed.....		70.3			74			17.5			61.2	



Date		Hartsville			Durango			Pima			Acala			Mebane			Lone Star		
		Flowers	Bolls shed	Bolls retained	Flowers	Bolls shed	Bolls retained	Flowers	Bolls shed	Bolls retained	Flowers	Bolls shed	Bolls retained	Flowers	Bolls shed	Bolls retained	Flowers	Bolls shed	Bolls retained
June 27					1	1	0				1	0	1	1	0	1			
28					1	0	1				0	0	2	1	1	1			
29					4	0	4				3	0	3	2	2	5	1	0	
30		1	0	1	3	1	2				4	1	3	3	2	2	2	0	
July 1		1	0	1	4	0	4				8	1	7	3	1	3	5	4	
2		2	0	2	5	1	4				6	3	3	10	8	2	6	2	
3		5	2	3	8	3	5				5	2	3	6	6	0	4	1	
4		7	3	4	3	1	2				9	7	2	8	5	3	6	3	
5		7	4	3	9	0	9				1	1	6	15	11	4	3	1	
6		12	6	6	3	1	2	2	1	1	5	2	3	15	9	6	7	7	0
7		13	4	9	6	0	6	4	1	3	8	3	5	20	13	7	7	1	6
8		12	4	8	7	5	2	11	2	9	16	3	13	5	4	1	10	4	6
9		14	5	9	2	1	1	1	0	1	14	12	2	18	6	12	12	2	10
10		14	6	8	12	6	6	10	0	10	22	8	14	14	7	7	27	9	18
11		26	12	14	8	5	3	8	1	7	21	12	9	29	21	8	19	9	10
12		21	7	14	19	14	5	9	0	9	14	10	4	26	21	5	17	9	8
13		40	26	14	13	9	4	17	3	14	39	31	8	52	42	10	49	30	19
14		27	9	18	21	14	7	19	1	18	36	31	5	33	24	9	35	24	11
15		29	20	9	13	9	4	17	1	16	20	15	5	33	31	2	25	22	3
16		35	20	15	29	25	4	17	1	16	32	24	8	45	42	3	46	40	6
17		38	28	10	26	20	6	16	1	15	32	27	5	39	35	4	26	22	4
18		45	32	13	21	16	5	29	4	25	35	23	12	28	24	4	48	37	11
19		25	18	7	27	20	7	12	4	8	23	16	7	50	39	11	49	41	8
20		51	38	13	50	20	30	26	2	24	52	37	15	63	45	18	54	44	10
21		37	28	9	21	11	10	21	4	17	39	24	15	44	18	20	43	22	21
22		39	28	11	20	9	11	29	6	23	53	22	31	43	20	23	58	23	35
23		46	32	14	31	16	15	31	1	30	50	26	24	42	23	19	55	34	21
24		34	28	6	27	21	6	20	4	16	48	39	9	46	21	25	73	47	26
25		45	37	8	34	25	9	36	7	29	54	42	12	43	31	12	57	51	6
26		50	39	11	46	31	15	22	5	17	70	61	9	52	45	7			

Sept.

TABLE VI.—*Number of flowers produced and bolls shed per day, and number of bolls matured by 25 representative plants of six cotton varieties in 1923—Continued*

Date	Hartsville			Durango			Pima			Acala			Mebane			Lone Star		
	Flowers	Bolls shed	Bolls re- tained	Flowers	Bolls shed	Bolls re- tained	Flowers	Bolls shed	Bolls re- tained	Flowers	Bolls shed	Bolls re- tained	Flowers	Bolls shed	Bolls re- tained	Flowers	Bolls shed	Bolls re- tained
Sept. 9.....	2	1	1	—	—	—	8	3	5	5	0	5	0	0	0	0	0	0
10.....	1	0	1	—	—	—	5	1	4	0	0	0	1	0	1	0	0	0
11.....	0	0	0	—	—	—	3	2	1	2	0	2	0	0	0	0	0	0
12.....	0	0	0	—	—	—	2	0	2	1	0	1	0	0	0	0	0	0
13.....	0	0	0	—	—	—	6	4	2	0	0	0	0	0	0	0	0	0
14.....	1	1	0	—	—	—	3	1	2	3	0	3	2	0	2	0	0	0
15.....	—	—	—	—	—	—	3	3	0	4	0	4	—	—	—	1	0	1
Total flowers.....	1,356			1,265			1,157			1,290			1,101			1,235		
Total bolls shed.....	987			895			339			925			803			919		
Bolls retained.....	369			370			818			365			298			316		
Per cent shed.....	72.8			70.8			29.3			71.7			72.9			74.4		

TABLE VII.—*Showing number of days after flowering at which bolls shed at the United States Field Station, Sacaton, Ariz., in 1922 and 1923*

Number of days after flowering	1922				1923					
	Variety				Variety					
	Acala	Du- rango	Pima	Lone Star	Acala	Du- rango	Pima	Lone Star	Harts- ville	Me- bane
1.....	1	4	4	2	5	2	—	4	3	7
2.....	9	32	—	3	16	11	2	10	9	10
3.....	41	60	—	16	36	36	14	17	29	13
4.....	101	94	2	29	97	81	17	73	60	54
5.....	251	186	12	83	134	158	39	128	126	110
6.....	319	186	16	142	169	165	37	159	190	148
7.....	284	199	15	124	169	132	30	183	200	172
8.....	166	119	16	110	85	81	31	120	130	165
9.....	74	64	10	46	47	64	21	66	73	38
10.....	32	52	18	36	44	38	21	48	44	43
11.....	31	36	13	11	32	25	10	23	22	20
12.....	16	27	12	14	20	21	22	15	25	11
13.....	12	14	13	6	20	21	13	16	13	13
14.....	5	19	9	5	11	7	9	10	13	10
15.....	5	8	6	2	4	14	13	8	8	5
16.....	3	7	4	6	6	6	13	3	4	3
17.....	5	6	6	—	7	2	7	4	17	5
18.....	3	2	1	1	2	9	4	6	—	2
19.....	2	2	4	1	4	3	4	2	6	1
20.....	1	3	3	1	4	5	5	5	1	2
Mean age at which bolls shed.	6.7	6.9	10.1	7.2	7.0	7.2	9.1	7.3	7.3	7.2

Data for two seasons on the number of bolls shed each day from the first to the twentieth day after flowering are shown in Table VII. While a few bolls were shed more than 20 days after flowering this was due to bollworm injury and is not included in the above table. Martin and Loomis found that with Pima cotton in Arizona the greatest shedding of bolls occurred between 4 and 14 days after flowering. It will be seen from Table VII that the average age at which bolls of the Upland varieties shed was about 7 days, while the average age at which Pima bolls shed was about 10 days in 1922 and 9 days in 1923.

TABLE X.—Showing the varietal differences in length of boll development period of 6 varieties of cotton grown under conditions obtaining at the United States Field Station, Sacaton, Ariz., in 1923

5-day periods (flower dates)	Mean number of days from flower to open boll					
	Hartsville	Durango	Pima	Acala	Mebane	Lone Star
June 27 to July 2	48.5±0.71	47.0±0.29	-----	48.1±0.30	48.3±0.32	47.3±0.80
July 3 to July 7	53.3±.47	47.4±.27	45.7±0.66	50.9±.58	50.2±.67	49.5±.71
July 8 to July 12	51.5±.19	46.4±.44	49.7±.47	48.5±.18	48.6±.22	49.2±.16
July 13 to July 17	50.8±.20	44.8±.45	53.6±.22	47.9±.52	48.2±.39	47.7±.26
July 18 to July 22	53.1±.23	45.7±.22	54.7±.24	48.5±.20	50.3±.30	50.3±.24
July 23 to July 27	52.7±.17	44.8±.22	57.0±.24	50.4±.31	49.6±.33	51.8±.22
July 28 to Aug. 1	54.8±.41	45.5±.28	57.5±.21	50.7±1.09	49.5±.77	52.0±.00
Aug. 2 to Aug. 6	57.4±.45	47.7±.33	60.8±.25	51.5±.33	54.4±1.12	54.2±.47
Aug. 7 to Aug. 11	59.2±1.18	47.4±.58	64.6±.25	56.6±.79	54.4±1.09	58.1±1.42
Aug. 12 to Aug. 16	61.0±1.91	52.0±.80	68.3±.33	60.8±1.66	59.0±.00	-----
Aug. 17 to Aug. 21	63.0±.00	53.0±.82	72.6±.45	-----	-----	-----
Aug. 22 to Aug. 26	69.0±.68	-----	79.7±.69	-----	-----	-----
Aug. 27 to Aug. 31	-----	-----	83.3±.41	75.5±.79	-----	-----
Sept. 1 to Sept. 5	71.3±2.46	-----	85.5±.01	74.8±1.32	-----	-----
Sept. 6 to Sept. 10	-----	-----	90.7±.86	79.0±.00	-----	-----

#### THE PERIODS OF BOLL DEVELOPMENT

Table X shows the mean number of days from flower to open boll of flowers produced from June 27 to August 31, grouped in five-day periods. It will be noted that in all varieties except Pima the boll development period was shorter for flowers that opened between July 13 and July 17, than for the periods immediately preceding and immediately following. This undoubtedly was due to the fact that the plants suffered from lack of moisture for a few days about September 1 to 4, causing some of the bolls to open prematurely. The Pima plants did not suffer, and the bolls did not show the reduced period of development.

Another decline in the boll development period, which also is probably due to a period of water shortage, is noticeable in the Hartsville, Durango, and Mebane varieties in bolls produced from flowers that opened between July 23 and July 27. If this interpretation is correct, such recording of the periods of boll development would be of use in cultural experiments in determining the conditions of growth, and whether sufficient irrigation water had been applied. The gradual lengthening of the boll development period as the season advances is in agreement with the results reported by King (3), Martin and Loomis (7), and Martin, Ballard, and Simpson (6). The mean period of boll development of the six varieties for the season of 1923 are shown in Table X.

#### COMPARISONS OF YIELDS

The cotton growers in the Salt River Valley are confronted with the problem of choosing the variety that will yield the largest returns under their conditions. In order to meet the higher fixed charges of irrigated farming and the higher cost of harvesting the cotton crop in this region, a larger return per acre must be assured than is necessary in the greater part of the cotton belt. The returns per acre derived from Pima cotton during the years 1916 to 1919, when it was the only variety grown, were undoubtedly greater than could have been secured from any other variety. In recent years, however, the market conditions have favored the Upland varieties, though opinion is much divided as to which Upland variety is the best for their purposes. Though the question turns largely upon the yields in the present state of the market it is recognized that premiums can be secured for Upland staples of good quality, and that it is desirable to unite upon a single Upland variety in order to develop local supplies of good seed.

TABLE IX.—Yield of seed cotton of the varieties compared in 1920, 1921, 1922, and 1923 at the United States Field Station, Sacaton, Ariz., given in pounds and by rows, and the total per plot

Row numbers	1920			1921			
	C2-13 Pima	C2-14 Durango	C2-15 Lone Star	C2-12 Pima	C2-14 Durango	C2-15 Lone Star	C2-13 Acala
1.....	64.8	52.5	59.5	80.6	39.5	61.8	61.1
2.....	63.1	51.1	66.4	78.5	28.6	57.4	64.3
3.....	74.4	49.7	64.2	78.7	20.2	66.9	67.8
4.....	59.3	47.9	63.7	66.1	24.6	61.7	64.1
5.....	63.6	46.0	64.4	67.2	22.7	67.7	65.0
6.....	72.5	49.4	64.4	65.1	22.6	65.8	76.7
7.....	58.8	63.7	58.3	62.9	34.1	78.1	79.4
Total.....	456.6	360.3	440.8	499.1	192.3	459.4	478.4

Row numbers	1922				1923					
	C2-14 Pima	C2-13 Dur- ango	C2-15 Lone Star	C2-12 Acala	C2-11 Pima	C2-10 Dur- ango	C2-14 Lone Star	C2-12 Acala	C2-9 Harts- ville	C2-13 Me- bane
1.....	88.5	59.3	48.4	62.8	80.5	70.6	71.4	91.2	77.3	78.1
2.....	69.8	54.4	58.2	58.4	85.0	82.1	84.4	83.8	81.8	74.4
3.....	70.8	57.6	48.2	58.0	86.1	82.6	81.0	80.0	77.3	83.6
4.....	64.2	45.9	47.7	59.6	82.7	85.6	93.7	90.5	78.7	95.5
5.....	70.4	57.7	48.9	60.8	81.8	72.3	106.3	80.4	76.6	88.3
6.....	65.8	53.0	46.4	60.6	95.0	92.0	87.9	90.8	90.7	84.8
7.....	82.9	58.0	55.7	51.6	80.8	92.6	64.5	84.8	94.3	92.9
Total.....	512.4	385.9	353.5	411.8	591.9	577.8	589.2	601.5	576.7	597.6

It will be observed in Table IX that Pima led all other varieties in yield during the first three years of the test. In 1923, a season which was very favorable for the production of all kinds of cotton, the Pima variety was third in rank from the standpoint of total production, though the differences were not significant in view of the wide variations between the individual rows.

The comparatively high yielding capacity of the Pima variety in some of the valleys of the Southwestern States is explained by the ability of this type of cotton plant to retain under normal conditions from 70 to 90 per cent of the total bolls produced. By referring to the data on shedding in Tables V and VI, and to figure 4, it will be seen that the Pima plants were able to retain and mature over 40 per cent more of the bolls than the best adapted Upland varieties grown under similar conditions in 1922 and 1923.

While the total yields of seed cotton have been larger for the Acala than for other Upland varieties during the three years in which this variety has been included in the comparison, the differences in row yields are so large that the totals may not be significant.

Greater irregularities appear in the yields of Durango and Lone Star than in those of Pima and Acala, and also wider variations in the rates of shedding. Such fluctuations may indicate a lack of stability in these varieties, as though less adapted than Pima and Acala to withstand the extremes of the summer climate.

In 1922 the behavior of the Acala and Hartsville varieties in late plantings was compared at Sacaton. For another purpose a very late planting of Acala was made, on June 22, at the side of a Hartsville planting on June 5. Even with this handicap of more than two weeks the Acala rows were nearly as productive as the Hartsville, as shown in Table X. In 1923 the two varieties were compared at different dates of planting, April 30 and May 20. The soil where these plant-

ings were made, in both 1922 and 1923, was somewhat impervious to irrigation water and impregnated with alkali, thus representing rather unfavorable conditions for crop production. The yields obtained in both years are given in Table X.

TABLE X.—Comparative yields of late planted Acala and Hartsville cotton varieties in 7-row plats at the United States Field Station, Sacaton, Ariz., in 1922 and 1923

Row No.	1922		1923			
	Date planted		Date planted			
	June 22, Acala	June 5, Harts- ville	Apr. 30, Acala	Apr. 30, Harts- ville	May 20, Acala	May 20, Harts- ville
	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.
1.....	16.1	14.4	21.3	12.7	29.4	16.5
2.....	12.7	14.0	27.5	17.0	28.3	16.9
3.....	13.6	15.1	24.6	19.1	32.4	22.2
4.....		17.3	24.1	19.8	25.8	23.0
5.....		14.1	22.9	29.8	31.8	23.0
6.....		15.1	24.0	31.7	21.6	18.2
7.....		7.4	22.8	24.6	27.9	13.4
Mean.....	14.13	13.91	23.90	22.1	28.17	19.07

#### BOLL, LINT, AND SEED CHARACTERS

Table XI shows data on the size of bolls of the varieties grown at Sacaton in 1923, as computed from three 20-boll samples of each variety. The figures give the number of bolls necessary to make a pound of seed cotton and the number of bolls necessary to make a pound of lint. A count was also made to determine the proportion of 3-, 4-, and 5-lock bolls produced by the different varieties. The Pima variety produced 91.8 per cent 3-lock bolls, 8.2 per cent 4-lock bolls, and no 5-lock bolls. Of the Upland varieties, Lone Star had 73.9 per cent 5-lock bolls, Mebane 72, Hartsville 64.3, Acala 54.4, and Durango 50 per cent.

TABLE XI.—Size of bolls, Sacaton, Ariz., 1923

Variety	Number of bolls per pound of seed cotton	Number of bolls per pound of lint cotton
Hartsville.....	60	234
Durango.....	76	266
Pima.....	122	451
Acala.....	63	200
Mebane.....	56	167
Lone Star.....	50	144

Table XII shows the variation which occurred in the lint percentage, lint index, and seed index for the different pickings. These figures are means of 20 representative samples from each picking. With all varieties except Hartsville the lowest lint percentage was obtained in the last picking. These figures show a tendency for both the lint index and the seed index to become lower toward the end of the season.

From Tables XI and XII it appears that Lone Star had larger bolls as well as a higher lint percentage and a higher lint index than any other variety, while the Pima had the smallest bolls and the lowest lint index. The largest seeds and the lowest percentage of lint were in Hartsville.

TABLE XII.—*Lint and seed data of five pickings as obtained from means of 20 samples from each picking, 1923*

Variety	Lint percentage				
	Mean first picking	Mean second picking	Mean third picking	Mean fourth picking	Mean fifth picking
Hartsville.....	28.1±.07	28.0±.24	25.3±.16	26.5±.15	26.7±.18
Durango.....	31.9±.10	31.0±.32	30.0±.08	32.3±.14	29.5±.17
Pima.....	28.2±.07	27.4±.07	28.5±.08	26.4±.15	-----
Acala.....	34.4±.13	32.5±.12	31.8±.13	33.1±.18	30.9±.21
Mebane.....	36.0±.15	35.9±.10	34.9±.11	35.7±.11	32.0±.23
Lone Star.....	36.4±.08	35.7±.07	35.4±.11	36.2±.13	32.4±.18
Variety	Lint index <sup>a</sup>				
Hartsville.....	6.37±.04	6.18±.04	5.59±.10	5.79±.05	5.56±.05
Durango.....	6.27±.04	6.26±.09	6.01±.04	6.00±.03	5.51±.05
Pima.....	5.46±.02	5.24±.03	5.50±.04	4.90±.06	-----
Acala.....	7.20±.07	6.71±.03	6.46±.04	6.50±.03	5.75±.07
Mebane.....	8.03±.06	7.95±.06	7.38±.04	7.58±.07	6.31±.07
Lone Star.....	8.62±.04	8.23±.06	8.29±.05	7.89±.07	6.71±.09
Variety	Seed index <sup>b</sup>				
Hartsville.....	16.3±.10	15.9±.14	16.4±.11	16.1±.10	15.3±.09
Durango.....	13.4±.12	14.0±.12	14.0±.10	12.6±.10	13.1±.10
Pima.....	13.9±.04	13.8±.07	13.8±.04	13.7±.08	-----
Acala.....	13.7±.08	13.9±.08	13.9±.10	13.1±.09	12.9±.11
Mebane.....	14.3±.07	14.2±.13	13.8±.09	13.8±.07	13.4±.06
Lone Star.....	15.1±.05	14.9±.09	15.1±.09	13.9±.11	14.0±.14

<sup>a</sup> Weight of lint from 100 seeds.<sup>b</sup> Weight of 100 seeds.

Fertile soils combined with the high temperatures which prevail over a long period, and the ample supplies of irrigation water which are usually available, often produce large, vigorous plants, with long branches and dense foliage, so that cultural precautions may be necessary to avoid too rapid and luxuriant growth. Larger yields are usually obtained from fields where the plants are 4 or 5 feet high rather than from taller plants. For good results the growth must be gradual and continuous instead of rapid and intermittent.

It is characteristic of Pima cotton to produce a strong upright central stalk with few vegetative branches and comparatively long fruiting branches, with long internodes and open foliage, which render it well adapted for conditions obtaining in irrigated regions (Pl. 1, A). Damage seldom results from lodging or breaking down of the plants unless they are extremely rank and widely spaced, so that the vegetative branches or side stalks grow large and are weighted with bolls.

The strong central stalk and upturned leaves are advantages of the Hartsville variety, though many growers would not consider an Upland cotton with a low percentage of lint (Pl. 1, B).

Durango, because of its upright habit of growth and strong, stiff central stalk, and rather narrow, deeply lobed leaves, has some advantages over the lower and more compact types of Upland cottons for growing under irrigation conditions (Pl. 1, C). Though over-rank Durango fields may be badly tangled, they are seldom lodged as flat on the ground as the Texas big-boll varieties. A tendency to excessive shedding under adverse conditions is responsible for occasional low yields as in the season of 1921 at Sacaton.

The Acala variety is somewhat like Durango in habits of growth, but usually with shorter stalks and less tendency to the "cluster" habit of short fruiting branches. The central stalk is fairly strong and the plants seldom become completely prostrate late in the season, even when heavily loaded with bolls (Pl. 1, D). Its ability to set bolls rapidly and to mature large crops under short-season conditions or when planted very late are important features.

The Lone Star and Mebane (Pl. 2, A and B) varieties representing the Texas big-boll type are very similar in their habits of growth, being lower and more compact than the preceding varieties, but with rather long spreading vegetative branches, short jointed stalks, large leaves and bolls. While these varieties often produce large yields under irrigated conditions, they frequently present difficulties in picking on account of the rather weak and flexible stalks which often lodge badly, so that many of the bolls lie on the ground where they may be mildewed or rotted, or the fiber stained by contact with soil.

Plats of the Pima, Hartsville, and Acala varieties are shown in Plate 3, A, B, C.

The average height of the 25 plants of each variety in 1923 which is shown in Table V represents fairly closely the relative height which plants of these varieties attain when grown on moderately fertile soils under irrigation.

### SUMMARY

Adjacent plantings of Pima Egyptian and several Upland varieties of cotton were made at Sacaton, Ariz., from 1920 to 1923, and data of plant behavior secured on habits of growth, flowering, shedding, yields, and lint and boll characters. A series of measurements on selected groups of 25 plants of each variety were conducted through the seasons of 1922 and 1923.

From these data it appears that the active growing and flowering periods of the Lone Star and Mebane varieties were much shorter than those of Pima, Hartsville, Durango, or Acala. The Pima plants continued growth over a longer period than any other variety, but in 1922 and 1923 had a somewhat shorter period of flower production than some of the Upland varieties.

The Upland varieties began flowering and fruiting earlier, and produced a greater number of flowers during the season than the Pima variety, but on account of more extensive shedding among the Upland varieties they brought fewer bolls to maturity. In 1922 the 25 plants of Acala produced a total of 1,948 flowers, which was the greatest number recorded for any variety, but only 579 bolls were retained, whereas 25 Pima plants produced 1,093 flowers, of which 902 became mature bolls.

The highest shedding rate, 74.4 per cent, was recorded for Lone Star in 1923, while the lowest rate was for Pima, in 1922, which shed only 17.5 per cent of the young bolls.

The low shedding rate of Pima enabled that variety to produce yields which compared favorably with those of Upland cotton, notwithstanding the more abundant flowering and larger bolls of the Upland varieties.

The average age at which bolls of the Upland varieties shed was about 7 days, while the average age of shedding for Pima bolls was 10 days in 1922 and 9 days in 1923.

The longest mean period of boll development recorded was 62.3 days, which was required by bolls developed from flowers of Pima cotton produced from July 6 to September 10, 1923. The shortest mean period was 47.1 days, which was recorded for Durango bolls developed from flowers produced from June 29 to August 21. The mean periods required for bolls of four other Upland varieties were Hartsville 54.3, Acala 51.5, Mebane 50.5, and Lone Star 51.0 days.

The Pima variety produced higher yields of seed cotton than any of the Upland varieties included in the test during the first three years. In 1923 there was no material difference in the yields of the varieties. The highest yield, 2,406 pounds of seed cotton per acre, was produced by Acala, followed by Mebane with 2,390 pounds, and by Pima with 2,368 pounds per acre.

Acala cotton produced somewhat higher yields than Hartsville when late plantings of the two varieties were compared in alternate plats.

By obtaining the weights of three representative samples of 20 bolls each from the different varieties it was determined that only 50 bolls of Lone Star cotton were required to make a pound of seed cotton, whereas 122 bolls of Pima were required. The number of bolls of Hartsville, Durango, Acala, and Mebane required to make a pound were 60, 76, 63, and 56, respectively.

Examinations of representative samples of the several varieties showed a range of lint percentages from 25 to 36 in the Upland varieties and from 26 to 28 in Pima. The lowest percentages were in Hartsville and the highest in Lone Star. The lint index or weight of fiber from 100 seeds was lowest in Pima and highest in Lone Star.

The strong central stalks and upright habits of the Pima, Hartsville, Durango, and Acala varieties are recognized as advantages in irrigated regions, where damage to bolls and difficulties in picking are often caused by the "lodging" or prostration of plants of varieties with lower and more spreading habits of growth, as Mebane and Lone Star.

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## PLATE 1

A.—An ideal Pima cotton plant grown at Sacaton, Ariz. Note the upright habit, scarcity of vegetative branches, scant leaf covering and long prolific fruiting branches, which make this variety well adapted to the irrigated regions.

B.—Typical Hartsville cotton plant grown under irrigation conditions in the Southwest. Note the stiff upright habit of central stalk and vegetative branches. These are recognized as advantages under irrigation conditions.

C.—Defoliated Durango cotton plant grown under irrigated conditions in the Southwest. Note the stiff upright habit of central stalk and vegetative branches.

D.—Defoliated Acala cotton plant grown under irrigation conditions.





## PLATE 2

A.—Lone Star cotton plant grown under irrigation conditions in the Southwest. Some of the bolls on the low limbs are often damaged in late season from being in contact with the wet soil.

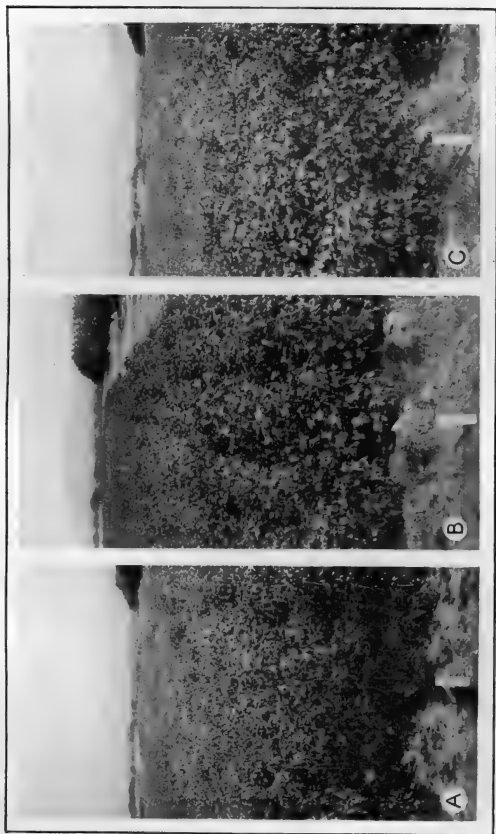
B.—Plant of Mebane cotton prostrated from weight of bolls late in the season. The tendency of this and other varieties with low growth habits to "lodge" when grown on heavy soils is regarded as a serious disadvantage for growing in irrigated regions.

**PLATE 3**

**A.—Plat of Pima cotton at Sacaton, Ariz., in 1923.**

**B.—Plat of Hartsville cotton at Sacaton, Ariz., in 1923.**

**C.—Plat of Acala cotton at Sacaton, Ariz., in 1923.**



# THE FUNGUS CAUSING THE COMMON BROWN ROT OF FRUITS IN AMERICA<sup>1</sup>

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Since investigators of the fungus causing the disease of fruits in the United States commonly referred to as "brown rot" have bestowed upon it several specific names, it seems worth while to determine, if possible, which name should be retained as the correct one. For many years the scientific name was generally accepted as *Oidium fructigenum* and later as *Monilia fructigena*, the American fungus being considered identical with the supposed single form of Europe, then passing under these names. As early as 1893 Schröter (11, p. 67)<sup>2</sup> by analogy had referred *Monilia fructigena* to the genus *Sclerotinia*, though he had not found an ascogenous stage and had no knowledge of its existence. Woronin (15) in 1900 showed that *Sclerotinia fructigena* is different from *S. cinerea*, the conidial stage of which was described by Bonorden (2, p. 76) in 1851 as *Monilia cinerea* and also referred to the genus *Sclerotinia* by Schröter (11, p. 67) in 1893. Since Woronin's conclusions were not immediately accepted, most investigators continued to use the name *Monilia fructigena* for the so-called brown rot fungus. After Norton (7) in 1902 proved that the species of *Monilia* causing the common brown rot of the peach in the United States is a conidial stage of a species of *Sclerotinia*, the name *Sclerotinia fructigena* came into general use. Later when Aderhold and Ruhland (1), Matheny (6), Conel (3), and other workers showed that the form common in America is not *Sclerotinia fructigena* but closely resembles and probably is identical with *S. cinerea*, the latter name became the one commonly accepted. Wormald (13), while studying the American and European forms, found that there were races or strains in *S. cinerea*. The American form being different from any of his European strains, he named it *Sclerotinia cinerea forma americana*.

Wormald (13) working with 13 strains of the American form found them to fruit more readily than the strains occurring in Britain and to show other slight differences when grown on artificial culture media. A constant but slight morphological difference was found in the conidial germ tubes. In the American strains these are at least 200 microns long before branching begins, while in the strains from Britain the germ tubes branch when quite short and are usually geniculate at one or more points. Wormald (14), has also recently described an ascogenous stage occurring in Britain that appears to be identical with ours, as indicated by his table showing measurements of asci and spores of both forms. In the same publication he states:

It seems certain, therefore, that the earlier records of the *Sclerotinia* in America were not of *S. fructigena* (Pers.) Schröter, and the more recent records show that the American form is one of which the conidial fructifications are indistinguishable from those of *Monilia cinerea* Bon. Of 13 strains of *Monilia* obtained by the present writer from various sources in North America, each has proved to be a fungus with a *Monilia* stage very similar to the gray *Monilia* common in Britain, but differing from the latter in its more copious

<sup>1</sup> Received for publication June 21, 1924—issued Nov., 1924.

<sup>2</sup> Reference is made by number (italic) to "Literature cited" p. 959-960.

development of conidia when cultivated on artificially prepared culture media, while strains of *Monilia* isolated from mummied plums obtained from France and Holland have proved to be culturally similar to the form occurring in Britain. The American *Sclerotinia* would appear, therefore, to be either a distinct species or at least a form culturally distinct from the European *Monilia cinerea* Bon. \* \* \*

Believing these differences to be sufficient to justify the consideration of the American form as a separate species, Norton and Ezekiel (8) have recently proposed the name *Sclerotinia americana* for the American fungus, thus raising Wormald's forma *americana* to specific rank.

In 1909 Pollock (9) called attention to the fact that Saccardo in volume 18 of the *Sylloge Fungorum* gives a description of *Sclerotinia fructicola* (Winter) Rehm, first described by Winter in 1883 under the name of *Ciboria fructicola*, from specimens on decayed peach fruits in North America, and later transferred to *Sclerotinia* by Rehm in a letter. He also states that as Winter's measurements of asci and ascospores correspond very closely to those given by Reade (10) for the ascogenous stage of the American fungus, Winter must have had this species. Pollock concludes:

If the rule of priority is to apply to the species name first associated with the perfect stage, the correct name of this fungus is *Sclerotinia fructicola* (Winter) Rehm instead of *Sclerotinia fructigena* (Pers.) Norton.

It should be remembered that at this time the American form was commonly assigned to the last-named species.

In 1920, Pollock,<sup>3</sup> at the annual meeting of the Botanical Society of America submitted data to show that the species of *Sclerotinia* common on American stone fruits is distinct from either *S. fructigena* or *S. cinerea* of Europe. Again he called attention to Winter's description of the American form in 1883 under the name of *Ciboria fructicola* and Saccardo's transfer of the species to *Sclerotinia* on the authority of Rehm. Winter's (12, p. 131) description is as follows:

*Ciboria fructicola* Winter nova spec. Cupulae gregariae, infundibuliformes, longissime pedicellatae, extus intusque brunnae, glabrae, usque 4 mill. diam., siccae margine involuto, extus griseae. Stipes concolor, flexuosus, usque 5 mill. longus, inferne fusco-villosus, ex epicarpio in corpore sclerotioideum fusco-atrum transformato oriens. Asci cylindracei, apice obtusi, deorsum attenuati, 8-spori, 130-160  $\mu$  longi, 8-8½  $\mu$  crassi, membrana ascorum apice incrassata et poro pertusa. Jod—. Sporae monostichae, ovoideae continuatae, hyalinae, 10-12½  $\mu$  longae, 4-5½  $\mu$  latae. Paraphyses filiformes, sursum parum incrassatae hyalinae, ascos aequantes. Ad Persicae vulgaris fructus putridos. Bethlehem, Pennsylvania, U. S. A. leg. E. Rau.

As indicated, Winter's material was sent him from Bethlehem, by Eugene A. Rau. Recently Mr. Rau has donated his collection of fungi to the Office of Pathological Collections of the United States Department of Agriculture. Through the kindness of Dr. C. L. Shear, in charge of that office, the writers have been permitted to examine what is evidently a part of the type collection of Winter's *S. fructicola*. The packet is labeled in ink "Peziza. On decayed peach in garden, May, '83," to which is added in pencil "new species Winter." The number, 37, also appears on the packet. The contents consist of portions of dried peach fruits and sclerotia together with dried and shriveled apothecia, which appear to be identical with those of the common American brown-rot fungus. Microscopical studies of this material were made by the junior author. The method used was as follows:

A small fragment of an apothecium was selected and placed in 95 per cent alcohol for a moment. It was then transferred to distilled water for several minutes. The purpose of the treatment with alcohol was to facilitate the absorption of water. For the purpose of expanding the material to as nearly normal size as possible it was placed in a drop of 7 per cent solution of potassium hydroxide on a microscopic slide. The material was then crushed and teased apart. The solution of potassium hydroxide was then withdrawn by means of a piece

<sup>3</sup> Also in a letter to the senior author.



of filter paper and a drop of stain solution (eosin or safranin) was added. Material treated in this way was examined with the following results:

Asci.—The asci are cylindrical to club-shaped with rounded apices. They taper gradually in the lower half to the point of attachment. The pore stains blue with iodine, and is brought out with great clearness by the use of this reagent. The 17 measured ranged from 117.8 to 161.5  $\mu$  in length and from 5.7 to 9.5 in width.

Ascospores.—Ascospores, eight in number, hyaline, nonseptate, in upper half of the ascus. They may be arranged end to end or, as in some cases, with their long axes at an angle to the long axis of the ascus, that is, seriate, ellipsoid in shape, some with quite pointed ends but generally with rounded ends, hyaline. Of 100 spores measured, the lengths ranged from 6.8 to 13.1  $\mu$ , with the greatest number 10.2  $\mu$ . The widths varied from 3.4 to 6.8  $\mu$  with the greatest number 5.1  $\mu$ .

Paraphyses.—Paraphyses filiform, hyaline, septate, ranging from 119.7 to 142.5  $\mu$  long and from 1.9 to 3.8  $\mu$  wide. The tip was rounded and in some cases slightly swollen. Some were branched. Only five paraphyses were measured, due to great difficulty in determining whether or not the ones seen on the slides were of full length or broken. The ones measured were quite certainly intact and of full length. For purposes of comparison the following measurements of *Sclerotinia* are given. It should be noted that these measurements, regardless of the names given, are of *Sclerotinias* from stone fruits in America.

Investigator	Date	Name given to fungus	Host	Asci	Ascospores
Winter...	1883	<i>Ciboria fructicola</i> .....	Peach...	130 to 160 $\times$ 8 to 8.5.....	10 to 12.5 $\times$ 4 to 5.5.
Reade...	1908	<i>Sclerotinia fructigena</i> .....	do.....	125 to 215 $\times$ 7 to 10.....	10 to 15 $\times$ 5 to 8.
Pollock...	1909	<i>Sclerotinia fructigena</i> .....	Stone fruits.	130 to 179 $\times$ 9.2 to 11.5.....	11.4 to 14.4 $\times$ 5 to 7.
Matheny.	1913	<i>Sclerotinia cinerea</i> .....	Peach...	135 to 190 $\times$ 6.9 to 10.5.....	10.5 to 14.5 $\times$ 5.2 to 7.5.
Matheny.	1913	<i>Sclerotinia cinerea</i> .....	Plum...	135 to 173 $\times$ 6.8 to 10.8.....	8.3 to 14.2 $\times$ 5 to 7.4.
Roberts...	1920	<i>Sclerotinia cinerea</i> .....	Peach...	152 to 176 $\times$ 8 to 10.....	6 to 15 $\times$ 4 to 8.
Dunegan.	1922-1923	<i>Sclerotinia cinerea</i> .....	do.....	130 to 186 $\times$ 5.7 to 13.3.....	9.5 to 14 $\times$ 5.7 to 7.6.
Dunegan.	1924	<i>Sclerotinia fructicola</i> ..... (Winter's type material.)	do.....	117 to 161 $\times$ 5.7 to 9.5.....	6.8 to 13.1 $\times$ 3.4 to 6.8.

The table shows a series of measurements, exclusive of Rau's material, in which the range for asci is from 125 to 215  $\mu$  in length and from 5.7 to 13.3  $\mu$  in width. For ascospores the range is from 8.6 to 15  $\mu$  in length and from 4 to 8  $\mu$  in width. These measurements agree fairly well with those of the type material of *S. fructicola*. That there should be some variations is to be expected when one considers the differences in the technique employed and also from the fact that Winter himself used dried material (Rau collected it in May, and Winter published his description in the September number of *Hedwigia* of the same year, namely, 1883), and that the measurements made by the present junior author were of material that had been dry for a little short of 41 years. It therefore seems certain that in 1883 Rau collected and Winter described the species of *Sclerotinia* which Norton in 1902 showed to be the ascogenous stage of our common brown-rot fungus.

Since, as previously noted, it has been well established that the name *S. fructigena* belongs to a European species that is distinct from the American, only the following binominals need be considered: *Sclerotinia cinerea* (Bon.) Schröter, *Sclerotinia fructicola* (Wint.) Rehm, and *Sclerotinia americana* (Wormald) Norton and Ezekiel.

Of these names *Sclerotinia cinerea* would be the correct one to apply to the American form, could it be shown to be identical with *S. cinerea* of Europe, if one believes it to be proper to choose the first-known name given to any form of the fungus, be it the ascogenous stage or a conidia-bearing stage, and properly published with an adequate description. If one believes that the first name applied to the ascogenous form and accompanied by an adequate description should be the one chosen, then *S. fructicola* would be the correct name both for the American form and the European form, again assuming their specific identity. If the American fungus is considered specifically distinct from the European, then the correct name is *Sclerotinia fructicola*, since, so far as known, it is the name first given to any stage of the fungus, the description is adequate, and it is also a name that should be acceptable to those who believe that the first name given the perfect stage should be chosen.

The name *Sclerotinia americana* is untenable whether or not one considers the American form specifically identical with the European. In the former case *S. cinerea* holds priority, while in the latter case *S. americana* is far antedated by *S. fructicola*.

Though at first the writers were inclined to believe there was sufficient evidence to justify consideration of the American form as specifically distinct from the European, further study has indicated that the differences, mainly physiological, are not sufficient to be regarded as specific. It seems best to regard the American fungus as a form or rather as consisting of a number of forms or races of *Sclerotinia cinerea*, accepting as the correct specific name the one first applied to the conidial stage of the European fungus.

Races or strains of the European form have been recognized and studied by Wormald (13), Killian (5), and others; of the American form by Wormald (14) and by Ezekiel (4). The writers have grown strains of the American fungus in parallel cultures with the European *S. cinerea* and have found the same differences noted by Wormald.

None of the American strains had quite the characteristically scalloped margins which the European form has when grown in plate cultures, but some of them had scalloped margins not greatly different from those of the European form (Pl. 1, A and B). The conidia of the American strains produced germ tubes which did not branch until they had become considerably longer than those produced by conidia of the European form, the germ tubes of which showed much branching shortly after their emergence. On the other hand, the strains of American origin when grown on an extract of dried peaches and subjected to the oxidase test, in accordance with a method described by Wormald (13), gave results which are in all respects identical with those produced by *S. cinerea* from Europe. The strains of the American form show great differences in their growth on artificial culture media. Although most strains when grown on 4 per cent potato agar produce an abundance of conidia, some strains produce conidia in abundance only for a short time. With these strains the production of conidia becomes less with each transfer, and finally only microconidia are formed. One strain obtained from a single ascospore has been grown by the senior writer for four years and now produces conidia even more profusely than at first. Moreover, it produces conidia on many media on which every other strain tried has produced none. The differences in growth on artificial culture media between a strain such as this and one which produces only microconidia are very striking (Pl. 2, A and B). The former forms a thin web over the surface of the medium, most of which is covered with numerous grayish masses of conidia. The latter slowly develops a thick white mat, having a powdered appearance caused by the profuse production of microconidia. The growth seldom covers more than a small portion of the plate or tube slant, possibly because

development is so slow that the medium becomes too dry for further growth. If differences in reaction to artificial culture media alone are to be considered of specific significance, then the American fungus should be regarded as made up of several species. If, in addition to these cultural differences, the slight morphological differences exhibited by germinating conidia be considered, the separation of the European and American forms into two species is much more defensible. From a practical point of view this is very objectionable, however, because it would make impossible the specific identification of any except living material. It may be that further investigations, particularly of the ascogenous stages, will reveal important morphological differences, but until such differences are noted, it would seem much more logical and much more practicable to consider the American form and the European form as a single species having the name *Sclerotinia cinerea* (Bon.) Schröt.

### SUMMARY

The common "brown-rot" fungus of America has been given at various times the following names: *Oidium fructigenum*, *Monilia fructigena*, *Sclerotinia fructigena*, *Sclerotinia fructicola*, *Sclerotinia cinerea*, *Sclerotinia cinerea forma americana*, *Sclerotinia americana*. By examination of type specimens it is shown that *Sclerotinia fructicola* (Winter) Rehm, described by Winter in 1883 under the name of *Ciboria fructicola*, is identical with the ascogenous stage of the American brown-rot fungus, thus sustaining the contentions of Pollock. If it is assumed that the American fungus is different from the European form commonly known as *Sclerotinia cinerea* (Bon) Schröter, then its name should be *Sclerotinia fructicola* (Winter) Rehm, the first name known to be applied to any stage of it and also the first name applied to the ascogenous stage. If the American form and the European are considered identical, then the name should be either *Sclerotinia cinerea*, if one believes the first known name properly applied to any stage should be adopted as correct, or *S. fructicola*, if it is believed that the first known name properly applied to the ascogenous stage should be considered the correct one. The known differences between the two forms are chiefly physiological as revealed by differences in growth on artificial media. It is shown that the American strains may show differences in their reaction to culture media at least as great as those between strains from America and Europe respectively. It is believed that at the present time the known differences between the American and European forms are not sufficiently great to warrant considering the two forms as separate species. The name *Sclerotinia cinerea* (Bon) Schröt. is preferred by the authors.

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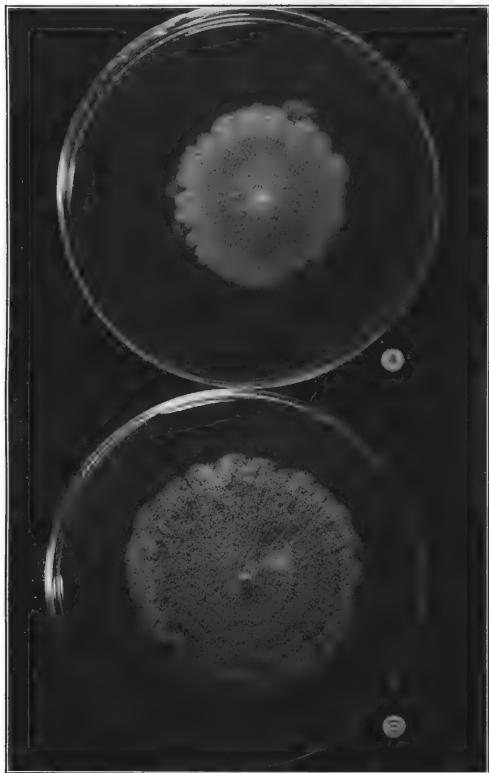
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## PLATE 1

Six-day-old cultures of *Sclerotinia cinerea* on 4 per cent potato agar plates.

A.—A culture of the fungus isolated from a mummied plum. England, 1920. Note the characteristically scalloped margins.

B.—A culture of the fungus isolated from a decayed peach fruit. Georgia, 1923. Note the scalloped margins resembling somewhat those of the European form, Plate 1, A.





## PLATE 2

Six-day-old cultures of *Sclerotinia cinerea* on 4 per cent potato agar plates.

A.—A culture of the fungus from a decayed peach. Georgia, 1923. Microconidia are produced in profusion. Note how little resemblance there is between this culture and those shown in Plate 1, A and B, and Plate 2, B.

B.—A culture of the fungus from a mummied plum. Virginia, 1921. The production of conidia is quite profuse.



# PRELIMINARY RESULTS WITH THE BORAX TREATMENT OF CITRUS FRUITS FOR THE PREVENTION OF BLUE MOLD ROT<sup>1</sup>

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Recent studies by the senior author on the effect of ultraviolet light in disinfecting the surface of citrus fruits, details of which will be published in the near future, have indicated that more than 99 per cent of blue-mold spores may be killed very quickly by light of short wave lengths, and at comparatively low cost, but that blue-mold rot is not proportionately checked. The two weak points in the ultraviolet treatment seem to be (1) the difficulty in killing the last small percentage of *Penicillium* spores, and (2) the lack of any later protection against stray spores that remain unkilld or may reach the fruit. Being thus strongly impressed with the desirability of using some agent that would have a continuing effect, the writers have undertaken the testing of a large number of mild chemical reagents with a view to finding inhibiting rather than killing substances. The stronger disinfectants would be objectionable, because their poisonous character would not permit residues to be left and because many of them would actually injure the surface of the fruit if not immediately washed off.

The general procedure has been to select chemicals for preliminary tests largely on the basis of cheapness and availability. Spores of *Penicillium* were treated with relatively strong solutions of these chemicals for periods of 1 to 24 hours, and were then plated to determine the killing effect. Parallel tests were made for inhibition. Any reagent that seemed at all promising was then applied to a few citrus fruits to determine whether it would injure the skin. If it seemed safe to use, an actual test was made to determine its protective action on severely wounded and inoculated fruit held under conditions of temperature and moisture favorable for blue-mold development. Usually some one of the stronger fungicides was included in each test as a basis for comparison.

Holding tests have been made thus far with fruit treated with solutions of the following chemicals: Sodium sulphate, sodium bisulphite, sodium borate, sodium fluoride, sodium perborate, sodium chloride, potassium permanganate, potassium borate, copper borate, bichloride of mercury, boric acid, salicylic acid, benzoic acid. Among these sodium borate, or borax, has seemed to give best protection against blue-mold rot.

The results of one holding test, made March 14, 1924, which included borax along with certain other materials will serve as an illustration and is shown in Figure 1. California navel oranges purchased in the Washington, D. C., market were used. The fruit was soaked for 10 minutes in inoculum carrying about 500,000 per cc. of mixed *Penicillium digitatum* and *P. italicum* spores. The fruits were dried, then atomized with the chemical solution (the control with water); dried again; punctured 100 times with pins to a depth of about half way through the peel. They were wrapped in waxed paper, packed in a tight

<sup>1</sup> Received for publication July 3, 1924—issued Nov. 1, 1924. This paper has been referred to the Bureau of Chemistry, United States Department of Agriculture, for consideration regarding the use of borax.

barrel with wet newspaper between each two layers of fruit; and were kept at room temperature of about 72° F. Examination for rot was made every three days. The results show some benefit from all of the chemicals; 1:5000 bichloride of mercury, 5 per cent boric acid, and 5 per cent sodium bisulphite had approximately the same effect; and 5 per cent sodium borate (borax) was distinctly

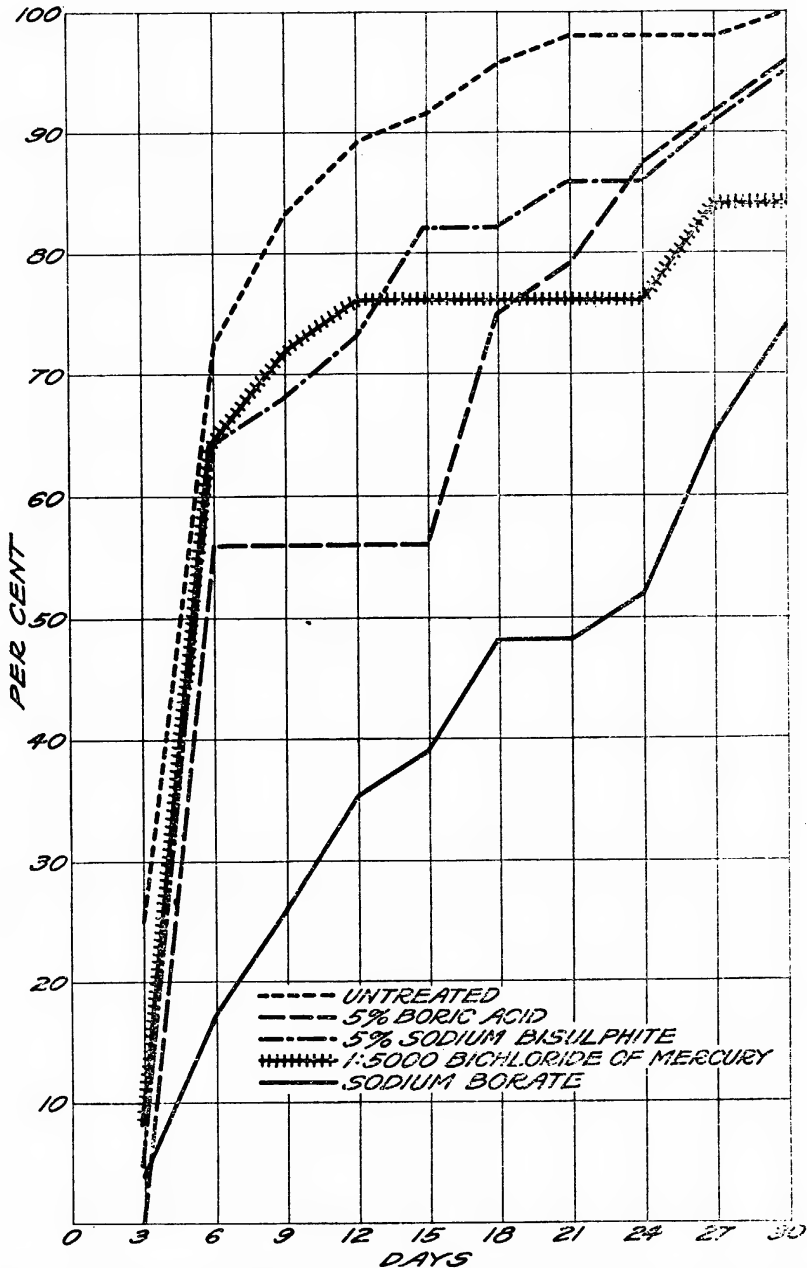


FIG. 1.—Percentages of blue-mold rot developing in oranges treated with various chemicals and those untreated during 30 days

more effective, particularly during the first 24 days of the test. In Figure 2 are shown the average results of 15 tests on oranges of 5 per cent borax treatment, as compared with no treatment, and 5 tests on lemons. These tests were made at various times. In all cases the fruit was punctured at 100 points, was inoculated with a suspension of blue-mold spores, and was packed between wet paper. The strength of the inoculum was varied in some of the tests, as was the sequence

of puncturing, inoculating, and treating with borax. The method of applying the 5 per cent borax solution was either by dipping the fruit momentarily in the solution, or by soaking for 1 minute or for 10 minutes, or by atomizing over the fruit, or by applying with a brush; all of these methods proved effective. The averages are based on 491 treated and an equal number of untreated fruit for

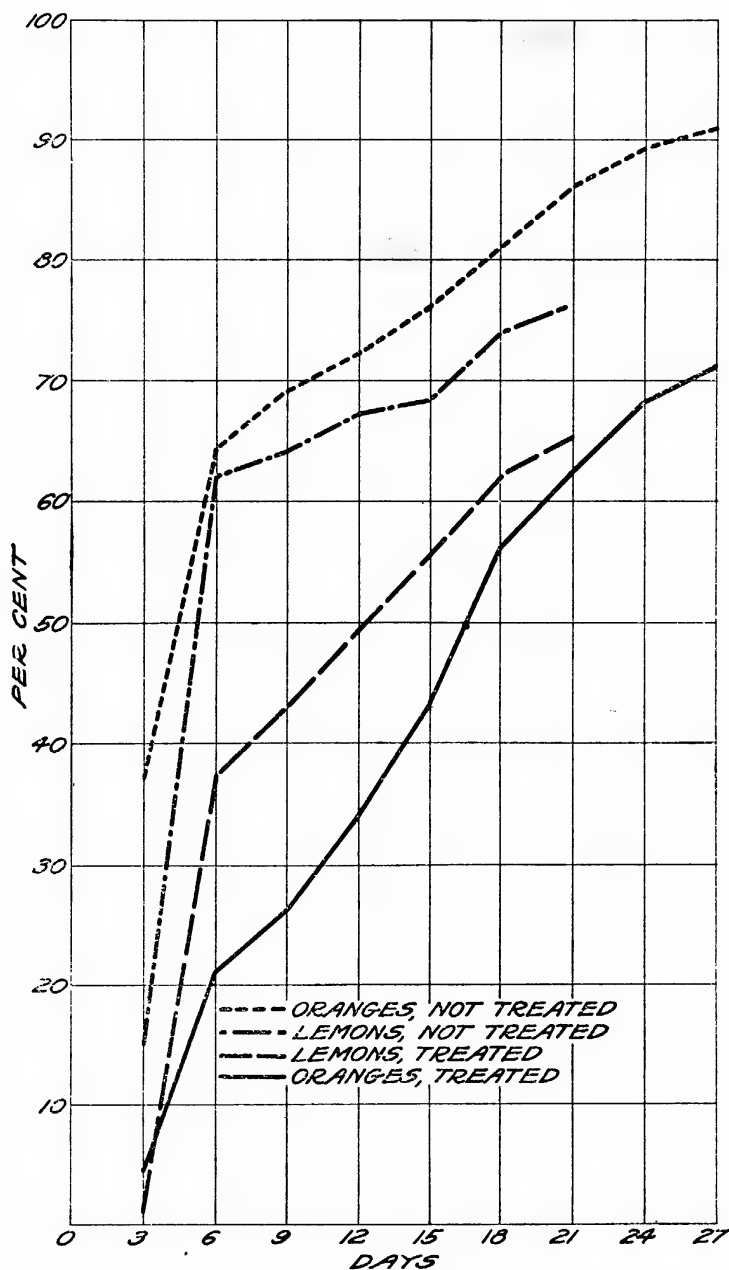


FIG. 2.—Percentages of blue-mold rot developing in oranges and in lemons treated with 5 per cent borax solution and untreated during 27 and 21 days

the oranges, and on 270 fruits for the lemons. They show the effectiveness of the treatment under conditions of infection far more severe than would occur in even the worst commercial practice. The treatment with 5 per cent borax solution resulted under these exceedingly severe conditions in a distinct reduction in blue-mold rot, but did not give what would be considered satisfactory control. The treatment seemed to be more effective on oranges than on lemons.

In certain other tests the fruit was not only punctured, but was severely bruised by dropping several times from a table to the floor, and several slices resembling very severe clipper cuts were cut from the outside of the peel. In such cases soaking one minute in 5 per cent borax solution had a very satisfactory protective effect, but the mere brushing on of the solution was less effective.

A preliminary test to determine the value of the borax treatment under commercial conditions was made in Florida with oranges. In one series of tests

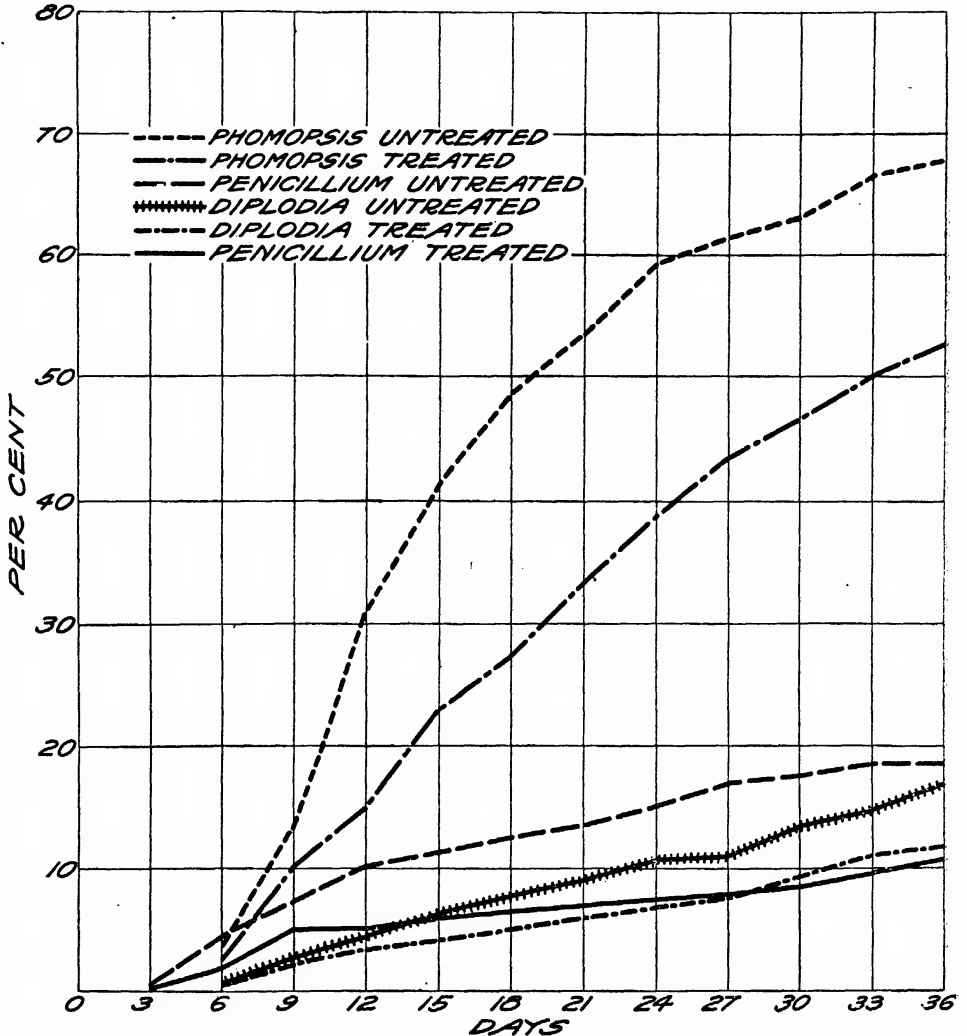


FIG. 3.—Development of Phomopsis stem-end rot, Diplodia stem-end rot, and blue mold rot (*Penicillium*) in oranges treated with 5 per cent borax solution and untreated during 36 days. The numbers at the left indicate the cumulative percentages of rot obtained as follows: For each period of three days the percentage of Phomopsis or of Diplodia or of blue mold rot is figured on the basis of the number of sound fruits in the test at the beginning of the three-day period. These percentage figures are added successively to give the cumulative numbers shown. Such numbers represent the proportion of rot that would develop if the test lot were restored to 100 fruits of like kind after removal of the rotten fruits at the beginning of each three-day period

samples of 100 fruit were taken from the packing houses; each sample was divided into two lots, one lot was dipped momentarily through 5 per cent borax solution and the control lot through water; the fruit was allowed to dry, and was held for several weeks at approximately 74° F. in a rather moist atmosphere. These conditions of temperature and moisture are more favorable for rot than usual commercial conditions, but were considered desirable in order to induce enough rot for a definite showing. In Figure 3 the averages are given for 11 sample lots of 50 oranges each, representative of Florida oranges in late April,

including Valencias in their prime and seedling oranges that were dead ripe. There is a distinct reduction in all three rots, *Phomopsis* and *Diplodia* stem-end rots showing decided reduction as well as blue-mold rot. This reduction in stem-end rots was an entirely unexpected outcome of the experiment. One possibility is that the borax solution may penetrate and disinfect the calyx edges or other portions of the button in which these two stem-end rot fungi are established, and from which they later extend their growth into the stored fruit.

Two commercial shipments of boxes of oranges and of grapefruit were made, one during April and one in May, 1924, from points in Florida in regular fruit cars under refrigeration to Seattle, Wash., and by ordinary express to Washington, D. C. Not enough blue-mold rot developed in either the treated or the untreated fruit for definite showing, even during a holding period of several weeks duration after arrival. The boraxed fruit in these shipments again showed a distinct reduction of stem-end rot when compared with the controls. Further tests will be made to determine the effect on blue-mold rot of the borax treatment in commercial shipments.

The question arose as to whether increasing the strength of the borax solution would give better control. At ordinary air temperature water saturation occurs at about 6 per cent or 7 per cent of borax, but solubility increases rapidly with rise in temperature. At 85° F. it is approximately 7 per cent; at 113°, approximately 14 per cent; and at 122°, approximately 18.5 per cent. In California lemons

and oranges are usually washed for several minutes in water between 115° and 120° for control of brown rot without bad effect. In the warm-water tests the writers have used 10 per cent commercial borax in water maintained at 122°, on plump but not turgid fruit that had been off the trees at least 10 days when purchased from the market. Seven tests, two with oranges and five with lemons, are averaged in figure 4. The percentages of rot are based on

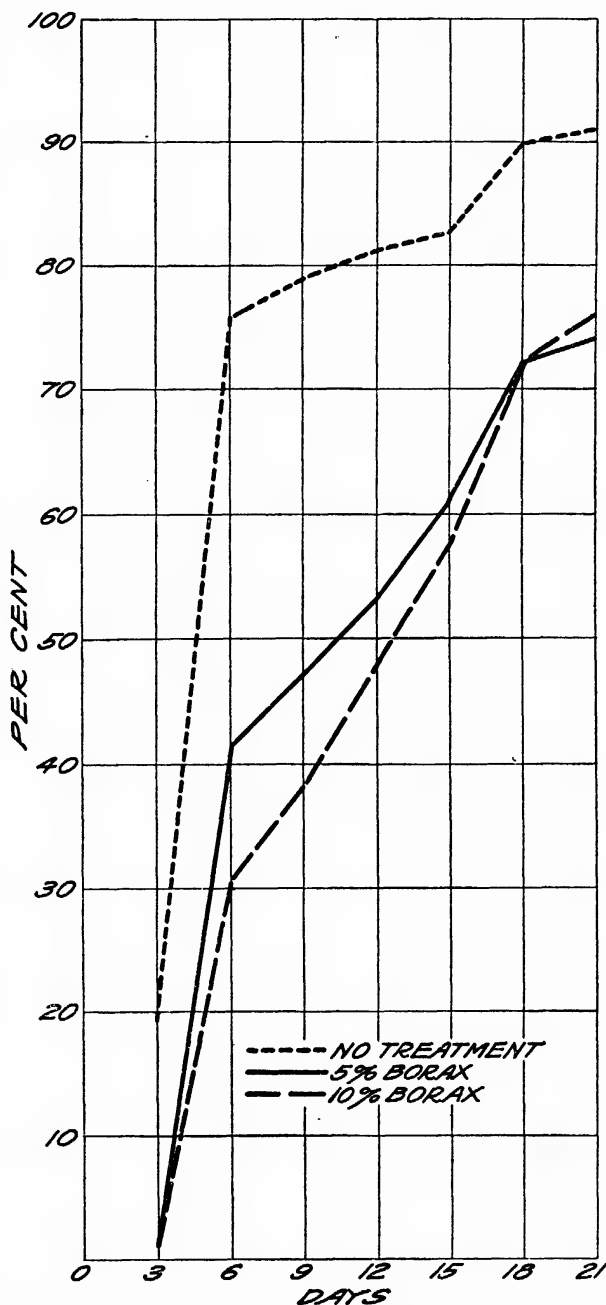


FIG. 4.—Percentages of blue-mold rot developing in citrus fruit treated with 5 per cent and with 10 per cent borax solution and untreated during 21 days

275 fruit originally given each treatment. There is some difference in favor of the 10 per cent as compared with the 5 per cent borax dipping treatment. It is possible that soaking tests of several minutes in the warm solution would show an increased effectiveness. The question requires further testing under commercial conditions of handling the fruit.

In packing houses having soaking-tank equipment a convenient place for applying the borax treatment would be in this tank. Usually the fruit

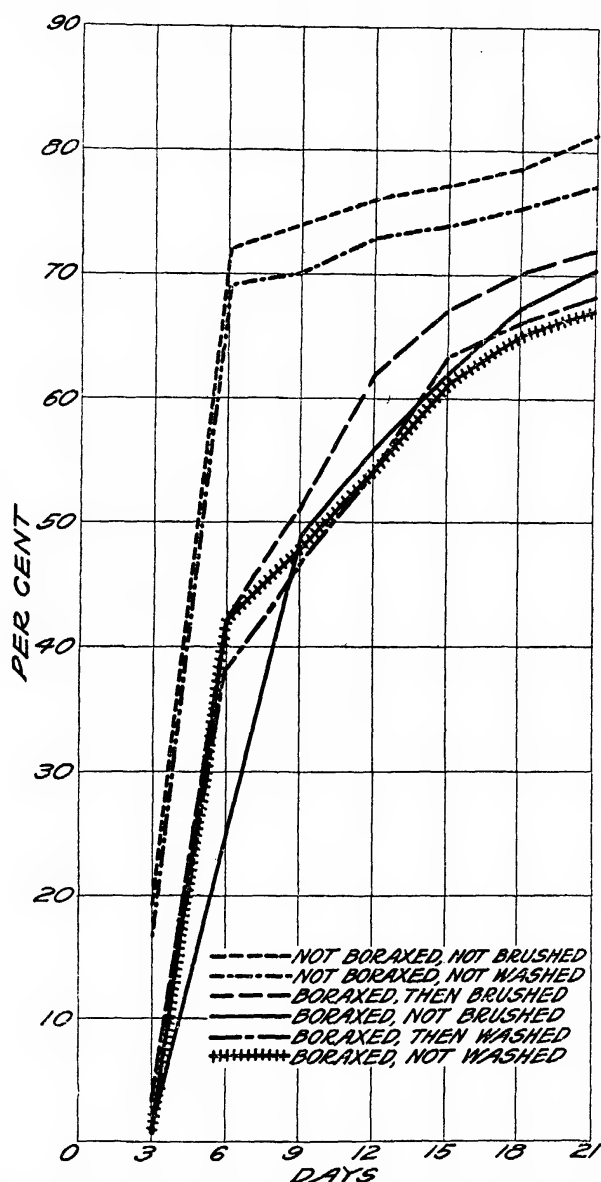


FIG. 5.—Percentages of blue-mold rot developing in citrus fruit treated with borax solution (two tests), treated with borax solution and later washed, treated with borax solution and later brushed, and untreated (two tests) during 21 days

passes from the soaking tank to scrubbing brushes over which streams of water play. These would wash off most or all of the borax. In the absence of a soaking tank, or for the purpose of leaving as much as possible to dry on the fruit surface, the borax solution might be played over the scrubbing brushes in small streams from perforated pipes leading from overhead tanks, or some atomizing device might be used just before the fruit enters the drier. Where polishing brushes are used after the fruit has dried, much of the borax coating would be removed. Tests were made to determine whether the removal of the borax coating, either by washing the freshly treated fruit or by brushing those on which the borax had dried, would materially lessen the protective effect. The fruit was heavily inoculated and was packed away between layers of moist paper at room temperature. Figure 5 shows the average results of seven separate tests, five with lemons, and two with oranges, totaling 340 fruits. The strength of the borax solution was 5 per cent for five of the tests and 10 per cent for two.

These results indicate that removal of the coating of borax really makes little difference. This is at variance with the working hypothesis on the basis

of which this work was undertaken. It may be that borax penetrates to some extent the tissues, especially at wounds, and thus has a residual effect even though the surface crystals are not present in quantity. However, one would naturally suppose that a surface coating of small crystals of an antiseptic substance that would readily dissolve in any moisture that would allow spore germination would give

added effectiveness in preventing such germination or subsequent invasion by the fungus mycelium. After the brushing the deposit of borax crystals remained, or was even increased in depressions, but was removed from the high places.

Practical packing-house tests will be required to determine the most effective and economical way of applying the borax treatment. From these preliminary laboratory tests it seems that the treatment would be about equally effective in any one of the several methods of application discussed.

Tests were made on the effectiveness of borax treatments at various intervals after artificial inoculation. The best results were obtained when the treatments followed immediately. With the lapse of two and one-half hours the treated fruit was no better than the untreated control. On the other hand, the tests with "commercially sound" fruit taken from packing houses, frequently one or two days after picking, indicate a material reduction in blue-mold rot and in stem-end rot, as is shown in figure 3. It is important to avoid delay in treating the fruit, and the borax treatment must not be relied upon to make up for careless handling. All the tests with severely wounded fruit indicate that the borax treatment will by no means prevent all of the rot. In general the laboratory tests had the advantage over carelessly handled commercial fruit of receiving the treatment promptly after wounding and had the disadvantage of being subjected to exceedingly severe wounding and holding conditions. The borax treatment should be regarded as a supplement to and not as a substitute for careful handling. It gives promise of having a place in protecting the fruit against a considerable proportion of the blue-mold rot that is likely to occur even with very careful handling, especially that incident during and after the packing process.

Borax has the advantage of being cheap and readily obtainable. It does not actively corrode metal and is not a poison. It is an article of common household use. The coating, even when freshly made with 10 per cent solution, is not very conspicuous on oranges and lemons; on grapefruit it shows at first somewhat more prominently as a grayish coating. After the fruit has been wrapped and packed and held for a week or more, one must look very closely to detect any difference in appearance between the treated and untreated fruit. It does not seem probable that the borax coating, even if all is left on the fruit, will detract materially from its appearance or affect its marketability.

As is well known, regulations of the United States Department of Agriculture, made under the pure foods act, prohibit the use of borax or certain other preservatives in food products and restrict their use on the surface of such products. In the case of citrus fruits the application of borax is made to the outside of the peel, a portion of the fruit that is not regarded as edible and that is discarded in the ordinary consumption of the fruit. If the fruit is washed before using, the adhering borax is readily removed. In commercial establishments for extracting juice and for manufacture of citrus by-products the general practice is to wash the fruit before using. In the making of citrus drinks the fruit is doubtless used sometimes without washing and the peel put in the drink. In this case there is considerable dilution with liquid. Our tests show that an orange of medium size, on an average, will remove 1 cc. of 5 per cent borax solution when dipped. Perhaps half of this drains off. On this basis a single orange might carry 0.025 gram (0.4 grain) dried borax on its surface. The therapeutic dose is given as 0.750 gram (12 grains), and is the equivalent of what might be carried on about 30 oranges.

Chemical analyses are being made by the Office of Soil Fertility Investigations of this bureau to determine the amount of borax present on and in the peel and in the edible pulp portion of oranges and of lemons that had been held 15 days

after treatment with borax solution of 5 per cent and of 10 per cent strength. Preliminary results indicate that the amounts present are even much smaller than those indicated and that the borax does not penetrate to the edible portion of the fruit.

No injury to the fruit through the use of borax solution has been observed. Most of the tests were made with fruit obtained on the market some time after picking, but a number of tests on grapefruit and on oranges were made with freshly picked fruit. The effect on very turgid fruit has not been fully tested.

#### SUMMARY

Preliminary tests have shown that commercial borax (sodium borate) solution in strength of 5 per cent or 10 per cent applied to the surface of oranges or lemons and allowed to dry greatly reduces blue-mold rot under experimental conditions that are unusually favorable for rot development.

Oranges and grapefruit handled in a commercial way, when treated with the borax solution, showed a marked reduction of both *Phomopsis* and *Diplodia* types of stem-end rot, as well as of blue-mold rot.

The borax treatment gives promise of being commercially valuable, in conjunction with careful handling of the fruit, in reducing losses from blue-mold rot.



# SNAILS AS PREDISPOSING AGENTS OF SUGAR CANE "ROOT DISEASE" IN LOUISIANA<sup>1</sup>

By R. D. RANDS

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United States Department of Agriculture*<sup>2</sup>

Root disease, or rootrot, has been considered a major factor in the gradually declining yields of sugar cane in Louisiana. The losses, particularly in "stubble," or ratoon crops, have lately attained such proportions as to render of questionable value the practice of growing even one year stubble. Although root troubles have in the past received but little scientific attention in Louisiana, rootrot in other countries has usually been ascribed to various fungi.

Close study of a large number of incipient cases in the principal sugar sections of Louisiana has revealed that a small snail (*Zonitoides arboreus*, Say)<sup>3</sup> may be primarily responsible for the trouble. So far as known to the writer, snail injury has not hitherto been reported anywhere on sugar cane. The damage, which is most extensive on the soft, white, terminal portion of the root, consists typically of minute cavities or pits 0.3–2.0 mm. deep and of similar diameter, resulting frequently in direct death of the growing point, and the development of secondary rootlets farther back. The latter, in turn, may be attacked, giving the roots a fibrous, bunchy appearance. On the older portions of the root the pits extend only to the hard central bundle sheath. No injury to the rhizome, or underground stalk, has been observed. Around the pits and abrasions lesions commonly develop, due to invasion of the weakened tissues by soil inhabiting microorganisms. The extension of these combined with further snail injury gradually brings about the final death of the root. In advanced cases most of the older, deeper roots have died forcing successive development of new ones nearer the surface of the soil. While root lesions apparently not connected with snail damage have been observed, most of the root deterioration seems to be directly proportional to the extent of such injury.

That the above-mentioned species of snail is responsible for the root pitting has been repeatedly confirmed both by direct field observations and by feeding tests with isolated snails in the laboratory. As many as 150 individual snails have been counted in casual examination of the soil about the roots of a single backward plant in a poorly drained field. They commonly occur in the earthworm

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<sup>1</sup> Received for publication July 28, 1924—issued Nov., 1924.

<sup>2</sup> Grateful acknowledgments are made to Messrs. D. W. Pipes, C. C. Krumbhaar, and Elliott Jones, managers of Southdown Plantation, Houma, La., for furnishing laboratory facilities and assistance in this investigation.

<sup>3</sup> The writer is indebted to Dr. Paul Bartsch, Curator of Mollusks, U. S. National Museum, Washington, D. C., for giving the following information:

"This mollusk is known as *Zonitoides arboreus* (Say). It has been reported from all the States of our Union, also from Canada as far north as Great Slave Lake. We have likewise had it reported from certain of the West Indian Islands. Normally, it makes its home under the dead bark of fallen trees and old rubbish heaps, squeezing into small crevices.

"Sugar cane, as I recall it, has quite a lot of small roots about its base, and drooping dead leaves hanging about the stalks, a condition not unlike a rubbish pile. It is, therefore, not surprising that we should find the organism in question in such locations. It may fall from these roots onto the soil or get down farther in the burrows of other animals."

tunnels and in the myriad other passages through the soil. Severely affected plants, when not killed outright in the early stages of growth, remain backward on account of their small and impoverished root systems, fail to tiller properly, and give at best an abnormally thin stand with consequently great reduction in yield. In certain of the older sections of the Louisiana Sugar Belt this year it is practically impossible to find a plant of the commonly grown varieties which does not show more or less of this root injury. However, in newly cleared land never before planted with cane, it is rare. Slight injury has also been observed on sorgo<sup>4</sup> and on maize, the latter being the only crop with interplanted cowpeas, commonly used in rotation with sugar cane.

Severe root injury and the same species of snail were also found on cane growing in the so-called "saw grass" or peaty soils of southern Florida. In the light sandy soils of the cane sirup sections of northern Florida and Georgia but slight injury was generally observed.

While the relative importance of primary and secondary agents in bringing about the final complex condition known as "root disease" in Louisiana is yet to be determined, severe root injury by snails is apparently a major predisposing and contributing factor.

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<sup>4</sup> This is more generally known as sweet sorghum.

# GREENHOUSE EXPERIMENTS WITH ATMOSPHERIC NITROGEN FERTILIZERS AND RELATED COMPOUNDS<sup>1</sup>

By F. E. ALLISON, *Biochemist*, and E. B. VLIET, *Assistant Chemist, Fixed Nitrogen Research Laboratory*, J. J. SKINNER, *Biochemist*, and F. R. REID, *Assistant Biochemist, Soil-Fertility Investigations, Bureau of Plant Industry, United States Department of Agriculture*

## INTRODUCTION

In connection with a study at the Fixed Nitrogen Research Laboratory on the possible transformation products of cyanamid,<sup>2</sup> there was obtained a number of materials and mixtures concerning which there is either very little known, or concerning which there is still some doubt as to their fertilizing value. Thus, in the preparation of urephos, a product was obtained in which approximately 55 per cent of the nitrogen was present as urea, 30 to 35 per cent as guanylurea, 5 per cent as ammonia, and the balance in undetermined forms, a small part of which was dicyanodiamide. There is very little data available on the use of guanylurea as a fertilizer, and the literature on cyanamide and especially on dicyanodiamide contains many conflicting statements. The experiments reported here deal with these nitrogenous materials, as well as others, such as sodium nitrate and calcium nitrate, included for comparison. In addition, a few experiments were included in which calcium sulphate, potassium sulphate, and acid phosphate, but no nitrogen compounds, were added. A portion (Table I) of the experimental work was conducted in 1919, but a later group (Table II) of pot tests was conducted during the early part of 1922.

## SOILS, PLANTS, AND FERTILIZERS USED IN THE EXPERIMENTS

The two types of soil used were the Norfolk sandy loam, a Coastal Plain soil obtained from near Norfolk, Va., and the Chester loam, a Piedmont Plateau soil secured near McLean, Va. Both soils are rather poor and typical of a large area along the Atlantic coast.

Wheat, beans, and cowpeas were grown. There was some hesitancy about using legumes in an availability experiment with nitrogen materials, but since legumes are widely different from wheat, it was decided to include them. The results with wheat and beans were satisfactory from the viewpoint of the experiment, but the cowpea developed so many nodules that it grew luxuriantly regardless of the nitrogen added. These results were therefore discarded. With the exception of potassium sulphate and calcium sulphate, which are chemically pure substances, the materials<sup>3</sup> used, together with their composition, are as follows:

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<sup>1</sup> Received for publication June 5, 1924—issued Nov., 1924. These experiments were conducted at the Arlington Experiment Farm, Rosslyn, Va., the Soil-Fertility greenhouses being used.

<sup>2</sup> Crude calcium cyanamid, not hydrated or oiled.

<sup>3</sup> The preparation of some of these materials is given in an earlier publication. See ALLISON, F. E., BRAHAM, J. M., and McMURTREY, J. E. FIELD EXPERIMENTS WITH ATMOSPHERIC-NITROGEN FERTILIZERS. U. S. Dept. Agr. Bul. 1180, 44 p., illus. 1924.

Fertilizer	Composition (per cent)	
	N	P <sub>2</sub> O <sub>5</sub>
Ammonium sulphate.....	21.02	-----
Sodium nitrate.....	16.16	-----
Calcium nitrate.....	12.55	-----
Urea.....	45.70	-----
Ammonium nitrate.....	33.93	-----
Ammoniated superphosphate.....	5.38	<sup>a</sup> 14.66
Cyanamid (unhydrated and unoled).....	20.27	-----
Urephos.....	6.30	<sup>b</sup> 3.66
Guanylurea sulphate.....	33.23	-----
Dicyanodiamide.....	66.43	-----
Acid phosphate.....	-----	<sup>c</sup> 16.12
Calcined phosphate.....	-----	<sup>d</sup> 17.53

<sup>a</sup> Solubility in 2 per cent citric acid; total, P<sub>2</sub>O<sub>5</sub>—17.45 per cent.

<sup>b</sup> Solubility in 2 per cent citric acid; total, P<sub>2</sub>O<sub>5</sub>—6.36 per cent.

<sup>c</sup> Solubility in ammonium citrate; total, P<sub>2</sub>O<sub>5</sub>—17.50 per cent.

<sup>d</sup> Solubility in 2 per cent citric acid; total, P<sub>2</sub>O<sub>5</sub>—27.16 per cent.

## SOIL PREPARATION AND FERTILIZATION

The sieved moist soils were weighed out into portions equivalent to 10 pounds of air-dried soil and the fertilizers thoroughly mixed with each portion. Two pots were used in each treatment. When the young plants were about a week old, they were thinned to 5 and 3 plants per pot, respectively, for the wheat and beans in the first experiment, and 10 plants per pot for the wheat in the second experiment. The general plan of the experiment is apparent from Tables I and II.

The quantities of fertilizers used were based on field applications, assuming 2,000,000 pounds of soil per acre to a depth of 6 inches. All pots, except those designated as "no fertilizer," received 80 pounds of P<sub>2</sub>O<sub>5</sub> and 40 pounds of K<sub>2</sub>O per acre in the forms of acid phosphate and potassium sulphate, respectively, except where otherwise noted. Ammonia was added at the rates of 10, 40, 80, and 160 pounds per acre. Where acid phosphate was used with cyanamid, the materials were applied separately. In some instances, calcined phosphate was used in admixture with cyanamid and also in one ammonium sulphate series for comparison. In the cases of urephos and ammoniated superphosphate (in the lowest application) acid phosphate was used to bring the P<sub>2</sub>O<sub>5</sub> content up to the proper amount. With the medium and heavy applications of ammoniated superphosphate, however, the P<sub>2</sub>O<sub>5</sub> contained in the material itself was in excess of the application decided upon. The nitrogenous materials were applied at various rates depending upon the material used. Adequate control pots were included in which applications of phosphate and potash were made, but no nitrogen.

## EXPERIMENTAL RESULTS

### AVAILABILITY STUDIES WITH BEANS AND WHEAT

The bean and wheat plants were harvested at the end of five and eight weeks, respectively. The relative green weights of the plants grown on the two soils with the different sources of nitrogen are summarized in Table I.

An examination of the table shows that ammonium sulphate, ammonium nitrate, ammoniated superphosphate, cyanamid, and urea all gave good increases in yields over the no-nitrogen fertilizer pots. The relative values of the different nitrogen carriers varied at the different rates of application and also varied with the soil and crop grown, as might be expected. The variations between the results obtained with the fertilizers used under a given set of conditions were,

however, not large but usually within experimental error. The comparatively limited data would not justify close distinctions between the values of the different forms of nitrogen. It is especially significant that cyanamid, which is ordinarily considered very slowly available and of questionable value when used in large applications in the field, proved to be very readily available under greenhouse conditions when thoroughly mixed with the soil. (See Pl. 1, A.)

The green weights secured with urephos were fairly good, but on the average poorer than with the sources of nitrogen discussed above. This was very likely due to the high content of guanylurea. Where the latter compound was used alone, it showed a marked retarding or toxic effect at the higher application, especially with beans, but there were no burning effects or dropping of the leaves noticeable. (See Pl. 1, B.) Where a mixture of urea and guanylurea nitrogen were in the approximate proportions found in urephos, the yields were the same within experimental error.

TABLE I.—*Summary showing green weights of beans and wheat grown with various sources of nitrogen, expressed on a percentage basis*

Source of nitrogen	Norfolk sandy loam		Chester loam	
	Beans	Wheat	Beans	Wheat

FERTILIZER RATIO a-1-8-4				
No nitrogen.....	100	100	100	100
Ammonium sulphate.....	110	127	118	122
Do. <sup>b</sup> .....	119	113	130	118
Ammonium nitrate.....		115		138
Ammoniated superphosphate.....	141	91	112	126
Cyanamid <sup>b</sup> .....	122	119	139	125
Urea.....	107	116	123	121
Guanylurea sulphate.....	99	103	101	101
Urea 70 per cent, guanylurea sulphate 30 per cent.....	125	121	104	128
Urephos.....	104	111	102	125
Dicyanodiamide.....	92	86	121	99

FERTILIZER RATIO-4-8-4				
No nitrogen.....	100	100	100	100
Ammonium sulphate.....	137	153	135	183
Do. <sup>b</sup> .....	174	142	158	186
Ammonium nitrate.....	144	133		206
Ammoniated superphosphate <sup>b</sup> .....	153	127	154	169
Cyanamid <sup>b</sup> .....	139	179	127	158
Urea.....	187	135	138	162
Guanylurea sulphate.....	91	98	110	131
Urea 70 per cent, guanylurea sulphate 30 per cent.....	128	148	139	172
Urephos.....		143		171
Dicyanodiamide.....	76	70	120	100

FERTILIZER RATIO-16-8-4				
No nitrogen.....	100	100	100	100
Ammonium sulphate.....	231	246	298	353
Do. <sup>b</sup> .....	238	217	300	352
Ammonium nitrate.....	280	167	266	342
Ammoniated superphosphate <sup>c</sup> .....	264	157	304	428
Cyanamid <sup>b</sup> .....	260	270	278	349
Urea.....	255	262	282	329
Guanylurea sulphate.....	77	108	71	175
Urea 70 per cent, guanylurea sulphate, 30 per cent.....	215	192	223	325
Urephos.....		187	182	311
Dicyanodiamide.....	42	44	80	96

\* The term fertilizer ratio refers to the percentage of  $\text{NH}_3$ ,  $\text{P}_2\text{O}_5$ , and  $\text{K}_2\text{O}$ , in the order named, as is customary in fertilizer practice.

<sup>b</sup> These pots received calcined phosphate.

<sup>c</sup> These pots received an abnormally high percentage of  $\text{P}_2\text{O}_5$ .

In connection with the study of urephos, pots were included to test the effect of calcium sulphate on plant growth. This material behaved as an inert material on these soils, even with comparatively high applications. This study was included because in the manufacture of a material such as urephos, where cyanamid is treated with sulphuric acid in excess, about 60 to 65 per cent of the fertilizer produced is calcium sulphate.

The results with dicyanodiamide show that it is quite injurious to both beans and wheat and not merely inert. Its toxic effects were much more pronounced on crops grown on sandy soil than on clayey soil, as is almost invariably the case with any injurious substance. Even with the lightest application, the bean leaves turned yellow on the edges and the tips of the wheat blades were burned. With the heavy application, growth was far below normal and the lower bean leaves dropped off. (See Pl. 1, C.)

#### AVAILABILITY STUDIES WITH WHEAT

A second series of experiments similar to those discussed above was conducted during the spring of 1922, using Norfolk sandy loam and Chester loam. The soils were not from the same lots as previously used, but were collected in the same general localities. Wheat was grown in all of the pots. The experiment was terminated six weeks after planting, earlier than intended because of a severe attack of mildew. The relative green weights of the wheat are reported in Table II.

The wheat data show no very marked differences in the case of the Norfolk sandy loam between sodium nitrate, calcium nitrate, and ammonium sulphate at the two rates of application. The slight differences are within experimental error. Cyanamid, used alone, was much poorer than either of these, but the different combinations with calcium nitrate were all in a class comparable with calcium nitrate alone, or with the two fertilizers used as standards for comparison. Replacing one-fourth of the cyanamid with calcium nitrate furnished a very satisfactory mixture, and where the two materials were used in equal parts the results were even better. Guanylurea sulphate was less satisfactory as a nitrogen carrier than any of the other materials, but did produce some increases, being about one-half as good as sodium nitrate at the largest rate of application and only slightly better than the control at the smaller rate.

The increases in the green weight of wheat produced by the fertilizers on the Chester loam were much smaller with all of the treatments than on the Norfolk sandy loam. In general, however, the results already noted in the case of the lighter soil were also obtained on the Chester loam with the one exception, namely, that the mixtures of cyanamid and calcium nitrate were no better than the cyanamid alone, at the largest rate. It is difficult to explain this result, considering that the nitrogen applications were rather small for greenhouse work and certainly not sufficient to produce any injury on this heavier type of soil.

The observations during growth agreed very well with the final green weights. Cyanamid was somewhat slower to act than ammonium sulphate and the two nitrates, but the mixture of cyanamid and calcium nitrate showed quick responses. Guanylurea sulphate produced no toxic effects, but merely failed to increase the growth to any marked extent.

The residual effects of the cyanamid applications on the two soils were negligible, the differences being within experimental error. The same was true of sodium nitrate at the two rates of application on the sandy soil, but on the loam there was a marked increase in yield over the control pots. Guanylurea sulphate increased the yield about 9 to 17 per cent on the sandy soil and 23 to 28 per cent on the Chester loam. These increases were much greater than with either

cyanamid or sodium nitrate, indicating that the material is slowly made available. This may explain the good results <sup>4</sup> previously reported, which were obtained with urephos on winter grain crops. In this case the fertilizer was applied in the fall and not used to any great extent by the plants until the following spring and early summer.

TABLE II.—*Summary showing relative green weights of wheat*

Source of nitrogen	Norfolk sandy loam		Chester loam	
	4-8-4 ratio	8-8-4 ratio	4-8-4 ratio	8-8-4 ratio
No nitrogen.....	100	100	100	100
No fertilizer.....	92	92	76	76
Sodium nitrate.....	216	309	137	158
Calcium nitrate.....	224	290	131	157
Ammonium sulphate.....	236	303	139	157
Cyanamid.....	175	234	125	135
Cyanamid 75 per cent, calcium nitrate 25 per cent.....	229	283	130	138
Cyanamid 50 per cent, calcium nitrate 50 per cent.....	240	303	128	128
Cyanamid 25 per cent, calcium nitrate 75 per cent.....	221	290	134	122
Guanylurea sulphate.....	117	200	110	128

## SUMMARY

The greenhouse experiments reported here, using Norfolk sandy loam and Chester loam soils, are too limited in extent to justify general conclusions, but do serve as good indications of the relative fertilizer values or toxicity for wheat and beans, of the materials studied. These indications may be summarized as follows:

The green weights showed no marked differences between sodium nitrate, ammonium sulphate, urea, ammonium nitrate, calcium nitrate, and ammoniated superphosphate. In the first experiment cyanamid also gave yields corresponding to ammonium sulphate used as a standard for comparison, but in a later experiment with wheat it was less satisfactory. This discrepancy in the results with cyanamid used under controlled conditions in the greenhouse merely verifies similar or more marked variations noted in the field.

Urephos was not so satisfactory a nitrogen carrier as the materials mentioned in the group above. The fact that from 30 to 35 per cent of its nitrogen is in the form of guanylurea is undoubtedly the explanation for the poorer showing. Although the results with the light and medium applications were rather good, the yields at the largest rate were considerably poorer.

Guanylurea sulphate is not a suitable nitrogen carrier. In some instances it produced fair increases in growth, while in others it either seemed to be inert or actually retarded plant growth to a considerable extent. There were some indications that the material is slowly made available and might be of use to a slow-growing crop, but further experimentation will be necessary to establish this point.

Dicyanodiamide injured the growth of wheat, causing a burning of the tips of the wheat blades and a retarding of growth. The injury was even more marked on beans, the leaves turning yellow even with the lightest application. With the heavy application, growth was very weak and the lower bean leaves dropped off. As is usually the case, the toxic effects were most in evidence on the lighter soil.

<sup>4</sup> ALLISON, F. E., BRAHAM, J. M., and MCMURTREY, J. E. FIELD EXPERIMENTS WITH ATMOSPHERIC NITROGEN FERTILIZERS. U. S. Dept. Agr. Bul. 1180, 44 p., illus. 1924.

# PLATE 1

A.—Growth of beans on a Norfolk sandy loam soil, receiving heavy applications of various nitrogen fertilizers. Acid phosphate and potassium sulphate were added to each pot, except the one containing cyanamid, to which calcined phosphate and potassium sulphate were added.

No. 1—No nitrogen; No. 2—ammonium sulphate; No. 3—urea; No. 4—urephos (Chester loam soil); No. 5—ammonium nitrate; No. 6—ammoniated superphosphate; No. 7—cyanamid.

B.—Growth of beans on Norfolk sandy loam, receiving guanylurea sulphate. Acid phosphate and potassium sulphate were added to each pot.

No. 1—No nitrogen; No. 2—small application of guanylurea sulphate; No. 3—medium application of guanylurea sulphate; No. 4—heavy application of guanylurea sulphate.

C.—Growth of beans on Norfolk sandy loam, receiving dicyanodiamide. Acid phosphate and potassium sulphate were added to each pot.

No. 1—No nitrogen; No. 2—small application of dicyanodiamide; No. 3—medium application of dicyanodiamide; No. 4—heavy application of dicyanodiamide.

(976)







# JOURNAL OF AGRICULTURAL RESEARCH

## CONTENTS

	Page
Individual and Age Variation in <i>Microtus montanus yosemite</i> - - - - A. BRAZIER HOWELL	977
A Genetic and Cytological Study of Certain Hybrids of Wheat Species - - - KARL SAX and E. F. GAINES	1017
Determination of Temperatures Fatal to the Powder-Post Beetle, <i>Lyctus planicollis</i> Leconte, by Steaming Infested Ash and Oak Lumber in a Kiln - - - - T. E. SNYDER and R. A. ST. GEORGE	1033
Bacterial Blight of Rye - - - - - C. S. REDDY, JAMES GODKIN, and A. G. JOHNSON	1039
Non-Inheritance of Terminal Bud Abortion in Pima Cotton - - - - THOMAS H. KEARNEY	1041
Relative Resistance of the Rice Weevil, <i>Sitophilus oryza</i> L., and the Granary Weevil, <i>S. granarius</i> L., to High and Low Temperatures - - - - E. A. BACK and R. T. COTTON	1043
Uninucleated Aecidiospores in <i>Caeoma Nitens</i> , and Associated Phenomena - - B. O. DODGE	1045

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## INDIVIDUAL AND AGE VARIATION IN *MICROTUS MONTANUS YOSEMITE*<sup>1</sup>

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### INTRODUCTION

While engaged in field work in Mono County, Calif., during July, 1922, the writer found that it was a year of extreme abundance for the common meadow mouse (*Microtus montanus yosemite* Grinnell), which inhabits the open meadow land of this region, and decided to make a detailed study of the individual and age variation occurring within this single unit group of this genus. A large series of specimens was carefully collected for this purpose.

It seems remarkable that despite the great activity displayed in the study of mammals in America during the past several decades no one has attempted a really comprehensive investigation to ascertain the amount of age and individual variation normal to any single group of small mammals. J. A. Allen (*2*)<sup>2</sup> published a short paper on the subject in 1894, and almost every reviser of a genus or group has done what he could to gain an understanding of these differences; but usually the material available has been of such a nature that no detailed and intensive study in this regard was practicable. Almost without exception series have been small, or, if large, the specimens were secured by different collectors; they came from different, even though closely grouped spots; their histories were not perfectly known; or they were collected with no view to a later study of a particularly intensive nature.

The purpose of the present contribution is twofold—to furnish a detailed report of the above nature and to offer a fairly comprehensive study of the osteology of the skull of a small rodent that is not particularly specialized. Only the exterior aspects of the bones are treated, with the purpose of filling a long-felt need that every systematic mammalogist has experienced at some point in his career. Throughout the discussion the writer has endeavored to treat fully all details that might be considered in any way important; but an effort has been made to avoid lengthy discussion of minute variations which are regarded as relatively unimportant and which would prove most tedious to the reader, and from which more pertinent matter might be separated only with considerable difficulty.<sup>3</sup>

<sup>1</sup> Received for publication Apr. 14, 1924—issued Nov., 1924.

<sup>2</sup> Reference is made by number (*italic*) to "Literature cited," p. 1015.

<sup>3</sup> The drawings, except those of the muscles and of the foot, were made by the writer. All details comprising each figure are enlarged in the same proportion, but there was no effort to have the various separate figures on the same scale. All numbers of specimens herein mentioned relate to serial numbers in the writer's private collection.

## MATERIAL

The series herein discussed is from Long Valley, Mono County, Calif. Here, at an altitude of 6,800 feet, Convict Creek meanders through miles of open, marshy, pasture land to join near by the upper reaches of Owens River. The conditions at this spot are very uniform. The trapping ground was far from any other association (except dry sage), and the growth, although exceedingly lush, consisted almost wholly of a half dozen predominating grasses and small sedges, with the local Iris in drier situations. As there are here few *kinds* of foods, there can not be great variation in the shaping influences that particular fare may have upon various parts of the skull. It is known that the stresses and strains developed by the different major muscles of the skull have the greatest influence upon its exact configuration, and much—perhaps most—of the individual variation therein is probably due to such cause. Where great diversity of food, such as grains, grasses, alfalfa, root crops, fruit, and the like, is available, there must be a corresponding diversity in the developmental stimuli of the skull muscles, according to whether an animal lives in a situation where one or another type of food predominates. In the Long Valley association such variety of food is practically at a minimum.

Disturbing influences as well are here very slight. The topography precludes the danger from severe floods, while the presence of the snow-fed creek, coupled with the fact that a part of its waters are so led as constantly to irrigate the meadows, minimizes the possibility of pronounced drought. Too, there is ample range for cattle in the valley, so that there is no overgrazing, with consequent destruction of favorable cover. Hawks are very few, and local indications suggest that the only furred predatory animals likely to exact appreciable toll upon the mice would be an occasional foraging coyote or mink. The only mammal which could compete with them ecologically to the slightest extent is the small shrew *Sorex vagrans amoenus*, which uses the runways of the rodents. Hence, theoretically, the meadow mice lead an existence that is unusually free from erratic influences, where all must eat substantially the same fare, and we are justified in assuming that there occurs less than the usual amount of individual variation among the specimens themselves.

From July 9 to 17, 170 voles were trapped within a territory extending along the bank of the creek for about one-third of a mile. Of these, all specimens with damaged skulls and a majority of the juveniles were discarded. Each specimen retained was weighed and carefully relaxed by manual manipulation before taking the three measurements of total length, tail, and hind foot. The mammae were then examined and notes taken on the number and size of the tubercles upon the soles of the hind feet. While skinning the specimens, observations were made upon the development and condition of both the outer and inner surfaces of the hip glands and upon the number and size of embryos when present. The skulls, after being labeled, were immersed in water to draw the blood, usually for 48 hours; the brains were then removed by syringing and placed in a 70 per cent solution of alcohol.

Forty-eight hours before cleaning, the skulls were placed in water, and were then heated in a solution of soap, soda, and ammonia, the cooking having been regulated with such care that it was possible to bring them to a point at which the saponified flesh could be removed by means of a fine stream of water without recourse to scraping or brushing, hence obviating the usual danger of damaging the finer processes while manipulating the scraper and brush. There has thus resulted a series consisting of 66 skins with skulls, besides a number of alcoholic specimens, collected within nine days in virtually the same spot by a single collector. In addition, every skull has been *thoroughly* cleaned and is perfect

down to the smallest detail (save for the lachrymals), except for four that were very slightly damaged by the traps.

Of this series but three specimens are definitely juvenile, the remainder being adults and subadults, all sexually mature. Such maturity can at once be distinguished in the field by an experienced collector, but not with certainty in the case of prepared specimens. Criteria are the general appearance of the animal before skinning, the condition of the pelage, and the development of the external genitalia. That such intangible points may be trustworthy in this regard is indicated by the fact that a majority of the very smallest females that are here designated as subadult contained embryos.

These 66 skins with skulls consist of 25 males and 41 females. Unfortunately no record was kept concerning the sexes of the discarded individuals, and it is now impossible to state whether the relative proportion of the two sexes in the series at hand is representative of conditions in the colony at large. The writer would be exceedingly loath to adopt the hypothesis that female *Microtus* substantially outnumber the males at birth, without evidence of a very conclusive character. Reference to field notes shows, however, that any disparity between the sexes in the series of different species of microtines that the writer has prepared has almost always been in favor of the females. This state of affairs can neither be ignored nor settled offhand.

#### METHODS EMPLOYED

In addition to extremely careful ocular comparison of the specimens, elaborate measurements of skulls have been taken by means of dial calipers of great accuracy. A list of skull measurements was selected which it was thought, after some trial, might prove to be of greatest value in the study of the subfamily Microtinae. These were made, tabulated according to sex, and arranged consecutively in descending sequence with reference to the condylo-basilar length as a basis. Taking the skull of the female with greatest condylo-basilar length as representing 100 per cent, and each of the other measurements of this skull as also representing 100 per cent, a list of percentages was made, tabulated, and plotted. It was thought that the actual plotting need be done only in the case of the more numerous sex. In the graph for body measurements and weights (fig. 2), also arranged according to the condylo-basilar dimension of the skulls, the averages for both sexes of the three body measurements and the weights have been taken as 100 per cent, each for its respective part.

This treatment of measurements—plotted by percentage and proportion—is believed to give a much clearer perception of the relation of each dimension to every other one considered than does the usual treatment of the subject. The one objection to this method is that in reducing, or rather magnifying, to percentage such small measurements as interorbital width, the coefficient of error is also necessarily magnified; but, nevertheless, such treatment allows one more easily to discern whether there be any correlation between the variations of the various portions of the skull.

It is perhaps too trite to be worth stating that no series of animals can ever be exactly uniform, for there exist too many developmental factors to permit precise correlation between parts, save in very rare instances; hence, all that is possible is an attempt to locate correlative trends between various portions of the animal.

In the accompanying graphs a mean or average line plotted between the perpendicular extremes would represent variation with age, while the extent to which the lines for each sex deviate from such an imaginary mean would signify individual variation.

## COLORATION

*Microtus m. montanus* and the subspecies *yosemite* average slightly darker than the general run of western meadow mice. The pelage is of the usual agouti pattern, but its precise color is not of interest here.

Although there is quite a bit of variation in the shade of the pelage among the males, this is not sufficient to make it easy to divide the series of this sex into two lots based on shade alone. They may, however, very readily be divided into two groups, one of which has an appearance rather dilapidated, this being in part due to wear, but to a greater extent to an uneven appearance which may be ascribed to fighting among themselves, as well as to the great prominence of the hip glands. The other group differs considerably in appearance, for although the glands in the larger individuals of this series are just as prominent as in some of the other lot, the skins have a sleeker look, are but little worn, and show practically no sign of fighting.

Of this second group, one specimen is a large, fully adult male in unworn-looking coat, but the remainder are smaller animals which are readily classed as subadults, sexually adult, except in three cases. These latter have a distinct look of immaturity, which consists of an appearance of shortness and compactness of the pelage of the entire body. The largest of these three, though still largely in the juvenile coat, is labeled as being sexually mature; but the other two are definitely juvenile. The pelage of none of these males is excessively worn.

Four of the females have the dorsal surface excessively worn, this being almost black in places. Severe wear results in the tips of a varying proportion of the hairs of the back breaking off, thus exposing to a greater or lesser extent the dark, plumbeous bases. There is considerably more variation of color in the remainder of the female than in the male series, and it is easy to divide them, according to shade, into two well-marked groups, although there are many intermediate examples the allocation of which is arbitrary. One group, very uniform, is of a brighter shade of brownish than the male series. A few of the males have counterparts in shade among the brighter females, but there are a number of the latter brighter than any of the males. The other group of females is exceedingly variable. Although it contains many individuals which might really, with equal propriety, be placed in the "bright-colored group," the series consists essentially of skins with a definitely more grayish cast, rather than a brownish one. The variation present is largely in the amount of gray. The larger, older females are for the most part paler and grayer than are any of the males and are rather uniform in this respect. The larger subadults are more variable, some of them being a trifle more brownish, while others are of a darker gray than the adults, due to an admixture of a greater number of black-tipped hairs. The whole "gray" series grades down to seven individuals which have the appearance of immaturity, being darker dorsally, with shorter, more compact pelage; but only the smallest specimen is definitely juvenile, for five of the remaining six contained embryos. There are a number of specimens, however, which, although with shorter head and body measurements than some of these six, have the type of pelage of the older animals.

In general, the largest specimens have the longest coats, but unless this be new, the hairs of the ventral surface are not relatively longer. Subadults in sleek pelage, even though this be rather short, normally have the longest hairs upon the underparts, and this surface is then correspondingly paler than in the case of individuals in which there has been wearing away of the pale hair tips. Similarly, specimens with palest underparts have a scanty growth of palish hairs upon the under side of the tails, which when worn away, leaves the tail almost black both above and below. The skin of the feet is uniformly sooty, but the hairs growing

upon these members are lighter and somewhat gray. This is longer upon the feet of fully adult examples than upon younger ones, thus causing the feet of mature animals to appear paler.

An arrangement of all males and females in two lots according to sex and with reference to the head and body measurement corroborates, on the whole, the above observations on coloration. The results, however, are not by any means all that could be desired, for probably 25 per cent of the specimens do not occupy the exact positions in the series where one considers, after a very careful study of the pelages, that they should belong. A regrouping of the skins with reference to the condylo-basilar length of the skulls gives results that are much more convincing, and there are then very few of the specimens which seem to belong in other positions. A third regrouping according to weight is a trifle more satisfactory still for males. Weights of females, however, can not be thoroughly uniform with development, because of the frequent presence of embryos. As the difference between these last two arrangements is practically nil, it is perhaps wiser to select the condylo-basilar criterion as the most desirable basis for grouping the skins according to age as indicated by pelage.

TABLE I.—*Measurement of males taken in the flesh, weights, and braincase capacity arranged in descending sequence according to the condylo-basilar measurement of the skulls*

Collection number	Measurements			Weight	Brain-case capacity <sup>a</sup>
	Head and body	Tail	Hind foot		
	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Grams</i>	
3835.....	128	53	23	67.0	130
3837.....	133	55	22	68.6	120
3812.....	133	47	23	70.7	109
3826.....	129	48	21	60.5	128
3853.....	126	47	23	59.8	114
3825.....	132	43	22	59.7	113
3854.....	127	47	22	55.6	106
3822.....	132	53	22	66.0	119
3818.....	134	( <sup>b</sup> )	21	61.4	113
3864.....	120	48	21	58.8	126
3846.....	118	49	21	51.0	110
3855.....	125	47	23	53.2	120
3848.....	115	55	22.5	45.3	119
3862.....	120	45	21	48.7	110
3883.....	120	53	22	49.8	118
3820.....	122	48	21	47.0	105
3841.....	113	48	22	42.2	104
3870.....	116	42	22	46.2	107
3847.....	111	49	23	43.7	116
3867.....	115	52	22	45.5	112
3887.....	110	46	22	35.6	110
3814.....	111	49	23	38.5	115
3849.....	111	47	23	43.8	106
3801.....	117	38	21.5	38.8	99
3857.....	97	41	22	26.5	96
Averages <sup>c</sup> .....	122	48.2	22	52.7	113.3

<sup>a</sup> In number of pellets of No. 10 shot.  
<sup>b</sup> Tail damaged: Measurement not considered.  
<sup>c</sup> Averages of 19 males, the largest two and smallest four specimens being excluded.



TABLE II.—Measurements of females taken in the flesh, weights, and numbers and size of embryos, arranged in descending sequence according to the condylo-basilar measurement of the skulls

Collection number	Measurement			Weight	Embryos	
	Head and body	Tail	Hind foot		Number	Size
	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Grams</i>		<i>Mm.</i>
3315.....	119	49	21	57.6	8	6
3356.....	124	46	22	49	-----	-----
3365.....	127	48	22	57.7	-----	-----
3319.....	121	45	22	56.4	-----	-----
3344.....	130	48	22	59	7	3-7
3371.....	128	50	22	53.6	-----	-----
3316.....	121	48	22	54.5	6	3
3345.....	119	49	22	48.4	-----	-----
3317.....	128	47	21	58.5	6	11
3334.....	123	46	20.5	49.6	-----	-----
3336.....	122	46	22	55	-----	-----
3381.....	121	47	21	55.9	-----	-----
3377.....	118	43	21	49.2	-----	-----
3363.....	128	40	22	62.2	8	11
3369.....	116	47	21	43.5	-----	-----
3376.....	123	44	21	55	6	10
3331.....	121	44	21	45.6	-----	-----
3321.....	118	48	22	45.2	5	2
3340.....	117	50	22	42.2	5	2
3380.....	118	47	22	46.4	-----	-----
3330.....	119	44	21	43.7	-----	-----
3327.....	120	47	21	55.1	5	13
3328.....	117	50	22	47.8	7	6
3372.....	118	45	22	41.8	3	9
3338.....	114	49	22	47	7	3
3350.....	117	50	22.5	46.5	6	5
3385.....	118	47	21	53.8	4	16
3386.....	117	47	21	46	5	3
3373.....	121	45	22	43.9	-----	-----
3313.....	118	45	21.5	36.2	5	2
3382.....	108	45	22	34.8	-----	-----
3339.....	106	47	21.5	38.8	4	2
3352.....	114	48	22	42.3	-----	-----
3329.....	118	47	21	42.7	-----	-----
3342.....	112	48	21	40.2	3	6
3361.....	105	46	21	40	-----	-----
3366.....	119	42	22	56.7	7	16
3351.....	115	47	21	48.2	-----	-----
3388.....	109	43	21.5	38.7	-----	-----
3332.....	98	39	21	29.6	-----	-----
3333.....	93	39	21	22.9	-----	-----
Averages <sup>a</sup> .....	118.2	46.5	21.6	48.2	5.6	6.9

<sup>a</sup> Average number of embryos of 41 females; average measurements and weight of 32 females; the largest three and smallest six being excluded.

CONCLUSIONS

The pelage of males is comparatively uniform, except that the older ones are rather ragged, chiefly due to fighting. The series of females is much more variable and may be divided into (1) a grayish group which, on the whole, contains a majority of the oldest individuals, many of which are grayer than any male; and (2) a brownish group composed chiefly of smaller subadults, but among which are a number of older individuals. The second group averages somewhat more brownish than the brightest males. Other things being equal, the larger and older the animal the longer the pelage. The feet of adults average paler than those of subadults. Juveniles are characterized externally by a slightly darker appearance and shorter, more compact pelage, although some of this indication of immaturity persists in sexually mature individuals.

A grouping of the whole series with reference either to weight in the flesh or condylo-basilar length of the skull gives results eminently more satisfactory and natural than when they are grouped according to the head-and-body measurement.

## MOLT

None of the specimens at hand is conspicuously in molt. In fact, a definite zone of molt such as often occurs in the case of *Thomomys* seems never to occur among microtines. The same applies to the writer's belief that there is no very definite season for the change of pelage in this subfamily. Individuals apparently molt largely according to age, although there are presumably other factors, chiefly climatic or dietetic, which may accelerate or retard such changes. About the time of sexual maturity there is usually a lengthening and slight brightening of the pelage, apparently due to a gradual growth of new hairs. This seems to begin upon the lower sides and extends upward. In some individuals it is also seen to extend from the face rearward but not at all pronouncedly. In the specimens that are most worn there is no evidence of the growth of new hairs, possibly indicating that such worn condition of the coat is not due to an imminent approach of the molt, but rather to some mechanical agency, or possibly to some pathological condition of the individual. Unfortunately, the writer failed to record, except in the case of a very few of the last specimens, the condition and color of the inner surface of the pelt. Such observations might have proved to be of decided interest.

## MAMMAE

All females had eight mammae, there being two pairs inguinal and two pairs pectoral. Some of these, usually the pectorals, were often extremely hard to distinguish in the smaller subadults. It is a well-known fact that not all the mammae may be functional in the case of young animals with small litters; hence the full complement may be correspondingly difficult of definition.

## PLANTAR TUBERCLES

It seems to have been accepted for years without serious question that species of the subgenus *Microtus* uniformly and invariably have six tubercles or pads upon the sole of each hind foot. The writer has found that there is much variation in this respect in the case of a number of species, though not all, and variation in the present form proves to be great.

Save in the case of spirit specimens, satisfactory determination of the plantar pattern must be made with fresh material. It is thus possible to compare only such unprepared animals as may be at hand, and it is impossible precisely to carry in one's mind the exact plantar development of the individuals in a considerable series. Hence, it is extremely difficult to record in satisfactory terms the variations which may occur. In the present instance the error must have been appreciable in judging the precise amount of development, whether one should say there were five or six pads upon an individual foot according to whether the sixth was vestigial, small, or medium.

There is considerable individual difference in the development of the tubercles which is entirely independent of age. In some animals they are relatively swollen and well developed while in others they are smaller, though more sharply defined as a usual thing. The general plan consists of a plantar pad upon the sole between, but posterior to, the bases of each pair of toes—four of them, which are designated the anterior ones. In addition, there is situated posterior to the first or antero-medial tubercle—the one between the bases of the hallux and second digit—a fifth tubercle, the postero-medial, which is normally the largest of all, but is variable to some extent. The usual sixth tubercle, the postero-lateral,

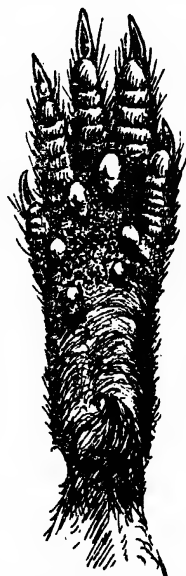


FIG. 1.—Sole of the left hind foot of *Microtus m. yosemite* (No. 3858), showing position of the plantar pads.

when present, is located posterior to the antero-lateral one and is the most variable of all. It may be entirely absent or may vary from vestigial to well developed. The writer's notes show that out of 72 animals preserved 19 had six plantar tubercles; 24 had five; 1 but "four and a half"; while in still another individual there were only four distinct pads, there being but the faintest trace of a fifth. In addition, one animal had six pads on one foot and five on the other. This great variation in plantar pattern is purely individual and probably indicates that the sixth tubercle is in process of being lost; although it is not impossible that the reverse contention is the proper one.

### HIP GLANDS

The same difficulty in a study of the hip glands is encountered as when recording data concerning the plantar tubercles, but to even a greater extent, for it is impracticable to examine the inner surface of the pelt of more than one or two animals at a time. This is a very real handicap, especially as the terms employed in describing the glands, such as faint, well developed, or reddish, are necessarily vague.

Externally the glands appear as a pair of patches situated well up on the hips and upon which the hair is more sparse, as well as shorter, than on the remainder of the dorsal surface of the trunk. In the dried skins of large males they are about 20 mm. apart, and as they are thicker than the remainder of the pelt they become considerably harder and less elastic to the touch when dry. These glandular patches evidently consist of groups of greatly developed and specialized sebaceous glands upon which the hair grows with lessened luxuriance. When they are prominent, there is a slight exudation of liquid from them—enough barely to moisten them—but this is not sufficiently oily to mat the surrounding hair to any extent. The writer has always considered that they act as scent glands and, judging from their situation, that they are for the purpose of leaving an odorous trail upon the closely encroaching sides of the grassy runways for the direction of other voles. This, however, is pure speculation. The inner surface of the pelts of old males shows scars about the head and shoulders. There is also a definite zoning of scars in the vicinity of the glands, indicating that these are probably particular targets for the ire of rivals. Similarly, some of the lagomorphs invariably endeavor to bite the testes of an opponent when fighting among themselves.

In a large male of the present series each hip gland measures about 30 mm. in an antero-posterior direction by 18 mm., and they are 20 mm. apart. Upon the inner side of the undistorted pelt they usually are situated wholly posterior to an imaginary line extending between the orifices in the hide originally occupied by the hind legs; but there is some variation in this respect and they may begin as far forward as a point half an inch anterior to such a line, thence extending rearward. When the glands first begin to develop there appears within the area a slight, whitish thickening upon the inner side of the skin, somewhat granular in texture. This increases in size and coarseness with age. In older males it becomes very thick and coarse, somewhat spongy, and at times congested with a conspicuous network of veins upon the inner surface. As this congested condition is found in some males but is absent in others, the writer judges that it not only indicates an increase in the activity of the gland, but that it may also be of a periodic nature and perhaps is dependent upon sexual activity, although this is merely an assumption upon his part.

The glands are usually of full size in adult males and only very slightly smaller in subadults of medium age, although they may be of distinctly finer texture and are probably much less active in the latter. It is odd that the only male of con-

siderable size which had the hip glands but very faintly indicated is the only specimen of the whole series that was in such condition as subsequently to stain the attached label with grease. Interesting inferences may be drawn from this case; but they are all pure speculation. All males except small juveniles have glands that are readily discernible from the inner surface of the pelt, and they apparently begin really to develop at about the time or just before sexual maturity is attained.

Fully adult females usually have hip glands almost as large, though of a finer texture and more poorly defined, than the males; and they never attain the congested condition often found in old examples of the latter sex. As a rule, an old female has about the same glandular development as a subadult male of medium size. There is some irregularity in its shape in females, however, and it may or may not be present to a slight degree in the smaller subadults of the female series.

The fact that both sexes of the present material have hip glands indicates that these do not constitute a secondary sexual character in the usual meaning of the term, although their development is probably directly contingent upon the attainment of sexual maturity by the animals. The definitely slighter development of the glands in females than in males should point to the hypotheses either that this was originally a race in which both sexes were equally well provided with glands, but that the females are now in gradual process of losing them, or else that the females were first glandless, but are now in course of acquiring them.

## EXTERNAL MEASUREMENTS

### LENGTH OF HEAD AND BODY

The measurement of total length of all animals in the present series was taken in millimeters after manipulation of the specimens so as to obviate to some extent the effect of *rigor mortis*. The measurement was taken in the usual way while stretching the body to a moderate degree, but with more than usual care.

Since collecting this series the writer has experimented with *Microtus californicus sanctidiegae* to ascertain the amount of variation that one might expect to occur in the measurement of total length when this was secured by a single collector. Two specimens were taken from the traps while still kicking and carefully measured as soon as movement had ceased. Their respective lengths were 199 and 178 mm. Twelve hours later, after a cold night and while the bodies were still very stiff with *rigor mortis*, they were manipulated to remove some of the rigidity before measuring; the figures then were 188 and 166 mm., respectively, while at noon, by which time the bodies were entirely relaxed, they measured 194 and 175 mm. It is thus seen that even when the measurement is taken carefully by the same person total length varies more than 5 per cent, according to the length of time that has elapsed between death and time of measuring. There must be at least 5 per cent additional difference in this figure when it is taken by several collectors, making in all a possible error of 10 per cent in the total length as given upon the labels of the average study skin. Add to this the fact that there is certainly more individual variation in this than in any other external measurement and it is evident that this character constitutes a very poor criterion by which to judge the age and development of a specimen. The length of head and body alone is much more reliable, for then there is not added to the coefficient of error for this, that for the length of tail which is especially subject to mutilation. The practice of taking total length, however, is so firmly established among American scientists that it would be difficult to

institute another mode. After all, it is a very simple matter, though perhaps less satisfactory, to ascertain the head-and-body length by subtracting that of the tail from the total length, as has been done in the present instance.

As mentioned previously, an arrangement of the series of skins with reference to the length of head and body does not give results that are as satisfactory as could be desired. Individual variation, of course, can not be escaped, but, still, this arrangement does not appear as natural to the orderly sequence of pelages and cranial development as does the arrangement according to the condylo-

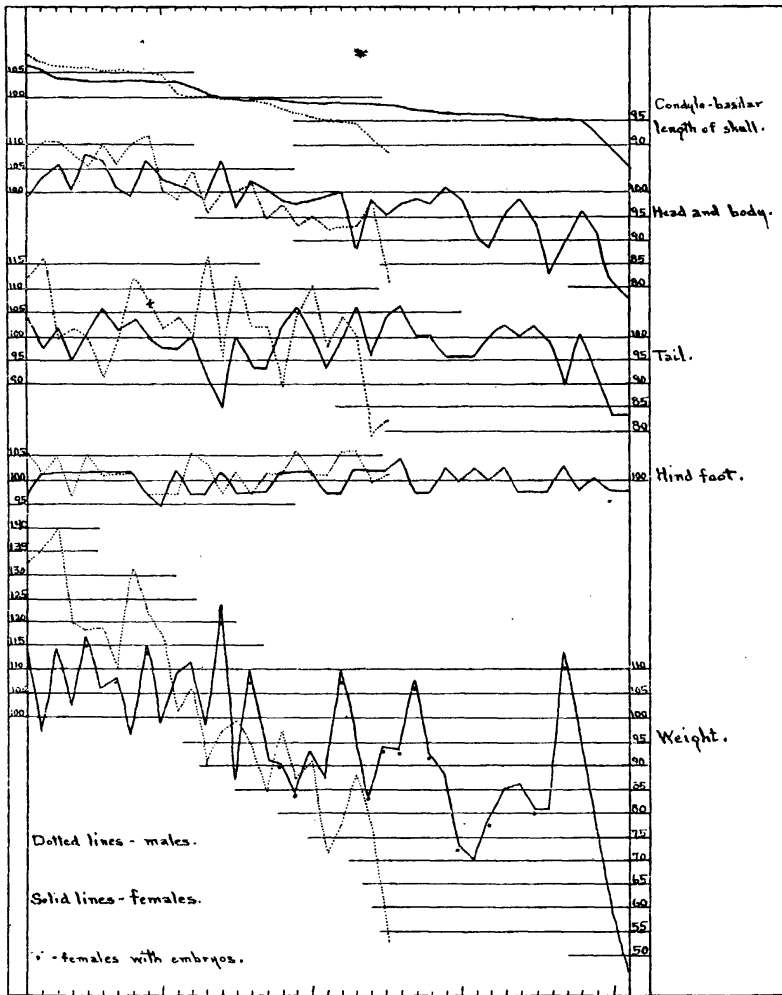


FIG. 2.—Graph showing external measurements and weights of both sexes of *Microtus m. yosemitae* reduced to percentages and arranged according to the condylo-basilar length of the skulls, with largest skull of either sex taken as par

basilar length of the skull. When thus disposed, the accompanying graph indicates that there is considerable individual variation; also that the average trend with age is for the head-and-body length to increase at a faster rate than the condylo-basilar length; in other words, the skull of the juvenile is proportionately larger with reference to the body of the animal than is that of the adult, roughly estimated 8 per cent larger. However, this relative disparity practically disappears as soon as sexual maturity has been attained. In taking the average of the external measurements and weights, several of the oldest and youngest specimens, judged by the condylo-basilar dimensions, were eliminated from consideration, even though there are but few in the present series. This was done for the purpose

of obtaining as nearly as possible a correct average of measurement for thoroughly normal, sexually mature individuals of the two sexes. The tables give information that may be desired concerning such specimens as might possibly be considered unusually large adults and juveniles.

For males the average head-and-body length is 122 mm., with extremes of 134 and 110—a difference of 24 mm., or 19.7 per cent of the average. The females average 118.2 mm., with extremes of 130 and 106, again a difference of 24 mm., or 20.3 per cent. The above figures indicate that males are consistently about 4 mm. larger in this measurement than are females, and the writer believes that this sexual difference in size is real, as it is corroborated by other data.

#### LENGTH OF TAIL

This measurement was secured by placing a millimeter rule at the upper side of the base of the tail of the unskinned animal and then bending this member at a right angle to the axis of the body, measuring to the tip but not including the terminal hairs.

In the case of *Microtus californicus sanctidiegae* it was found that there may be as much as 5 per cent variation in this measurement, depending on the length of time after death that it was taken. Add to this another 5 per cent to account for the different results obtained by various collectors, as well as the fact that throughout life the tail tip is especially subject to injury, and it is evident that this dimension is none too reliable. Extreme variation occurs even in the case of females. In males this measurement should be considered with decided caution, because the tails are so often injured and materially shortened while the animals are fighting among themselves. The average length of tail in males (with oldest and youngest specimens eliminated) is 48.2 mm., with extremes of 55 and 42—a variation of 13 mm., or 27 per cent, of the average. For females these figures are 46.5 mm., with extremes of 50 and 40, showing a variation of 10 mm., or 21 per cent. This difference of 6 per cent in the amount of variation in the two sexes may be partially ascribed to the greater propensity of the males to injury. In both sexes the tail averages 39 per cent of the head and body. Age causes very little variation from this figure. In the case of the six youngest females the percentage rises to 41, but this difference is almost too slight to be taken into consideration.

#### LENGTH OF HIND FOOT

There is probably less error in measuring the length of the hind foot from the heel to the tip of the longest claw than in either of the other two body measurements. There is little effect of *rigor mortis* upon this member; hence for all practical purposes the same figure will result regardless of how long after death the measurement may be taken, and there need be no difference in the results obtained by various collectors. The normal error in making this measurement, if half millimeters be recorded, will be about 0.4 mm., or slightly less than 2 per cent. In figure 1, of course, this possible error for individuals is magnified almost five times, although the average for the series is not affected.

There is considerable individual variation in the size of the foot, but practically none with age in the specimens at hand. In fact, the 12 largest males have feet that average a shade shorter than those of the 12 smallest; but this difference is so slight (0.1 mm.) that it is of little significance. It will thus be seen that full growth is attained by the feet at an early age, and for this reason foot length often is of much value in identifying juveniles of different species.

The hind foot of males averages 22 mm. in length, with extremes of 23 and 21, a variation of 9.1 per cent of the average; that of females, 21.6 mm., extremes

being 22.5 and 20.5, or a difference of 9.2 per cent. These figures are real and not fortuitous, for there are seven males with a foot length of 23 mm., but no female with feet so long. The individual variation in foot length is considerable, and this measurement may be of only slight diagnostic value when but a few specimens are available; but in series it is important.

#### LENGTH OF EAR

It is a number of years now since the writer became convinced of the worthlessness of the measurement of length of ear when taken from the fresh specimen in the field. A little experimenting demonstrated that without some sort of apparatus giving an even pull, it is practically impossible for a collector to arrive at any degree of accuracy or uniformity in taking length of "ear from crown," and "ear from notch" is almost as bad in this respect. In fact, the writer obtained some startling results, due to the facility with which the ear tip may be stretched from the head, and he thereupon abandoned the practice of taking this measurement.

In the present study the writer endeavored to use some sort of ear measurement taken from the dried skin, but, because of the usual distortion of this member while drying, has concluded that no reliance can be placed upon any figures resulting, and that it is better to omit this. A careful examination of the ears of the skins at hand indicates that there is no appreciable variation either individually or due to age.

#### WEIGHT

In any consideration of the weights of mammals one must remember that he is dealing with a measurement of mass, in three dimensions really, whereas all other measurements taken are of one dimension only. It is thus inevitable that there will be variation of a sharper degree than is usual in measurements of length; but this in itself is no argument against the importance and value of recording weights.

It is not usual for the Microtinae to lay up a great store of fat; in fact it is only rarely, if at all, that any definite quantity of fat is to be found upon them. For this reason the weights of individuals of like size do not fluctuate so decidedly as is the case with mammals which habitually take on a great supply of fat in anticipation of a period of hibernation. The weight of a *Zapus* taken in fall would be out of all proportion to that of an individual of like age taken in spring. The weight of this sort of mammal is thus of real value only when accompanied by data concerning its condition. The meadow mice of the present series were very uniform in this respect, and almost all contained a moderate quantity of food without having the stomachs greatly distended.

The individual variation in the weights of males is not excessive, considering that this is a measurement of mass. Variation with age is much more pronounced, of course. The average weight of 19 males is 52.7 gm., with maximum of 70.7 for old ones down to 35.6 for small young adults. This is a variation among sexually mature examples of 35 gm., or 67 per cent of the average weight. This amount of variation between individuals of such ages is undoubtedly excessive for rodents in general, but it is a well-known fact that it is usual in this subfamily. In fact, it may sometimes be greater, for veritable giants among meadow mice are not infrequently encountered, although there is none in the series under discussion. It will be noted that the largest male is considerably heavier than any of the females, even than those containing large embryos.

The weights of females are much less reliable and are more variable individually than those of the opposite sex because of the presence of embryos in a large number. The average weight of the 32 females is 48.2, with extremes of 62.2 and 34.8, a difference of 27.4 gm. This size variation among examples that are sexually mature of 56.4 per cent of the average is considerably smaller (11 per cent)

than for males, and if allowance is made for the fact that the three heaviest females were pregnant, the true variation due to age is yet smaller. As the average weight of females is considerably augmented by the presence of large embryos the true difference between the average weights of the two sexes must be considerably greater than the 4.5 gm. shown by the tables.

It is readily apparent that weight is to a large extent correlated with both length of head and body and condylo-basilar length of skull, especially in the case of males; but there is less connection in the female series, complicated as are the weights of many of them by the presence of embryos. There are many exceptions to this rule, however, constituting the normal individual variation.

### EMBRYOS

The period of gestation for *Microtus*, as determined by Bailey (3) in the case of the species *pennsylvanicus*, is 21 days. For the first few days after impregnation the embryos are so small as to escape detection by the average collector, who gives but a hasty glance into the abdominal cavity. The writer has no means of knowing the exact time at which appreciable swellings within the uterus indicate clearly that the animal is pregnant, but it is certainly more than three days. If we say three days, to be conservative, then only six out of seven pregnant females are recorded as in this condition by the careful collector. In the present series of 39 sexually mature females, 19, or roughly one-half, were definitely with young. It is entirely possible for meadow mice to bring forth a litter regularly about every 20 days, as determined by Bailey, and although such fecundity is doubtless exceptional in the wild state it probably is closely approached when a period of optimum population among the meadow mice is drawing near. The writer presented data elsewhere (5) which have caused him to believe that during the latter stages of and immediately following a peak population of small rodents which fluctuate markedly in numbers during certain periods there is a definite reduction in reproductive power. Indications in the present case, therefore, are that the females were bearing young at neither the maximum nor minimum rates and that the figures here presented are very likely average for meadow mice during midsummer.

The measurements of embryos given are merely for the length of the membranes in which they were contained, not for the total length of the fetus. These measurements vary from 2 to 16 mm., the latter figure being for examples at practically full term and which would probably have been born within 24 hours. Female No. 3844 contained 7 embryos, some of which were 3 and others 7 mm. in length, this variation probably being the result of some pathological condition. The average number of embryos for the pregnant females was 5.6. The 9 largest pregnant specimens contained an aggregate of 56 embryos, or an average of 6.2 each, while the nine smallest held only 44, an average of 4.9. This difference in fecundity according to age may be fortuitous but is probably not, for it is in line with what is known of the subject from experience with domestic animals. The weight of the eight embryos, practically full term, of female No. 3868 (an alcoholic specimen) was 17.3 gm. Only 8 of the 19 largest females contained embryos, while 11 of the remaining 20 subadults were in this condition. These figures may indicate a slight waning of the fertility of some of the oldest individuals.

### MYOLOGY OF THE HEAD

The writer thoroughly believes that the development of the skull after birth is chiefly influenced directly by the muscles and their mechanical effects while exerting stresses upon their insertions upon the bones; hence indirectly by the character of the food and feeding habits (including manner of mastication) of the animal.



The juvenile skull is the product of heredity, and there are undoubtedly present developmental factors of one sort or another which contribute toward general trends in certain directions; but the muscles exert powerful strains which can not fail to have the most profound influences in shaping the bones of the young animal. For this reason it is deemed impossible to gain a correct comprehension of the interrelationships and correlations of the different bones of the skull without some understanding of its major muscles, their attachments, and func-

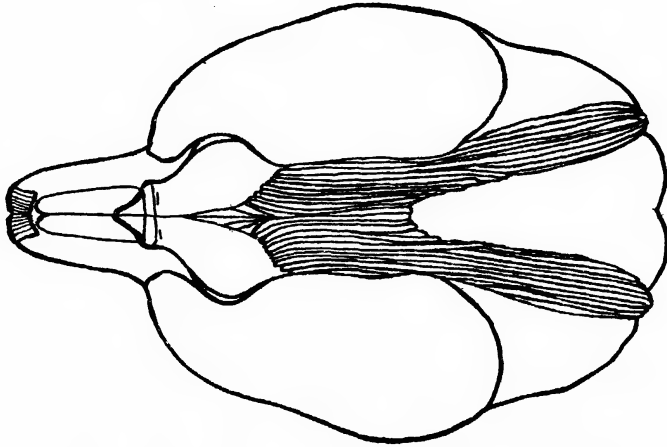


FIG. 3.—Head, showing position of the digastric muscle

tions (1, 4, 6, 8, 9). The interrelationship of these muscles is so complicated, especially in rodents, that only the main function of each muscle is touched upon here. Although this is not the place for a detailed treatment of the myology of the head, a brief description of the chief muscles used in mastication is deemed

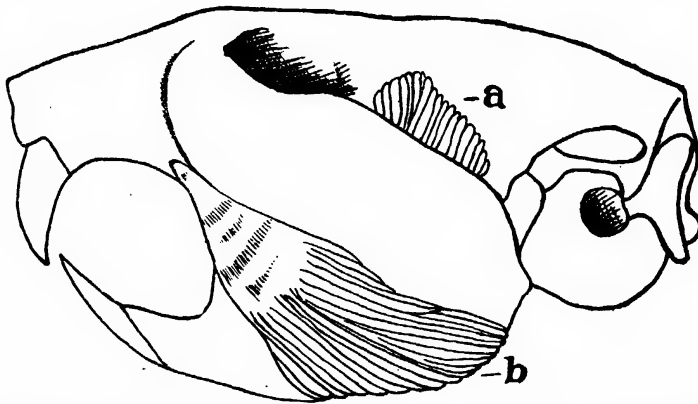


FIG. 4.—Head, showing (a) superior attachment of the deep part of the temporalis, the superficial part having been removed; and (b) the masseter superficialis

advisable. A discussion of the specific development of these as compared with the musculature of other species and genera of the Microtinae would be out of place in the present paper (7, 8).

**DIGASTRICUS.**—This arises from the posterior portion of the symphysis of the rami of the mandible, as well as for a millimeter or two upon the inferomedial part of the bone immediately posterior thereto, and extends as a slender band upon either side to insertions upon the paroccipital processes. Its functions are to draw the mandible backward and to open the mouth forcibly, although the latter action ordinarily requires very slight muscular force.

**MASSETER.**—The chief muscle of the skull and the one most used by the majority of mammals during mastication is the masseter. It is a compound muscle conveniently divided into three parts as follows:

**MASSETER SUPERFICIALIS.**—This arises from the slightly defined, small process directly below the anteorbital fossa (see fig. 4, *b*), in a stout aponeurosis, which rapidly broadens to form a thin band, developing muscle fibers and extending obliquely rearward to an insertion upon the inferomedial edge of the angular process of the mandible and the portion of the ramus immediately adjacent. The fibers superficial to this insertion are in turn inserted after the usual fashion in the intersection between this muscle and the pterygoideus internus. It is used in drawing the mandible forward.

**MASSETER ZYGOMATICA.**—This arises (see *b*, fig. 5) by a superficial fascia and deeper muscle fibers upon the inferior portion of the zygomatic arch from the anterior to the posterior angle, but additional strength is secured in this species by the extension of the aponeurotic sheet a couple of millimeters still farther forward along the zygoma, and to this extent it directly overlies a portion of the superficial division of the masseter major. It then descends with a posterior

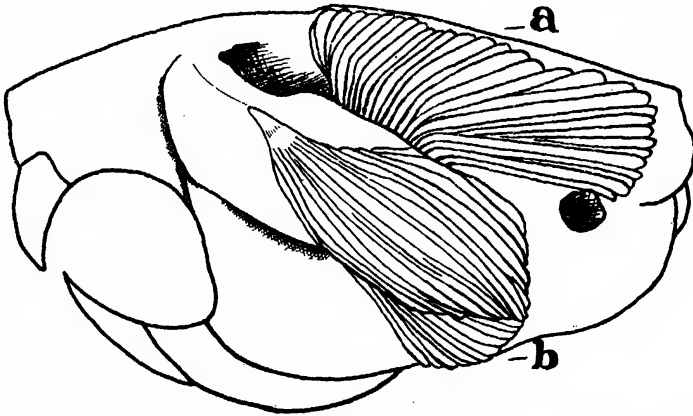


FIG. 5.—Head, showing (*a*) superior portion of the superficial part of the temporalis; and (*b*) the masseter zygomatica (masseter superficialis removed)

trend to an insertion upon the lateral face of the angular process, while a small portion is inserted upon the superior part of the medial face of the same. This muscle is used in closing the jaws, and probably to some extent in pulling the mandible forward.

**MASSETER MAJOR.**—This is properly divisible into two portions, the *pars superficialis* and the *pars profundus*. The former (see fig. 6) arises from the superior edge of the maxillary root of the zygomatic arch, covering the whole anterior face as far as the anterior angle of the zygoma. Its forward bulge covers the anteorbital fossa. It descends with a slight posterior inclination to an insertion upon the masseteric ridge of the mandible. The deep part (see fig. 7), considerably less in mass, arises within the orbit from the posterior edge of the maxillary root of the zygomatic arch, extending backward all along the superomedial edge of the zygoma. There is also a small division, ostensibly of this muscle (the exact status of which, in myomorphine rodents, is a matter of controversy), that arises from the anteorbital fossa and extends through the adjacent foramen to join the main muscle. This deep part is thinly inserted upon the mandible in a line extending from a point upon the lateral face of the ramus directly anterior to the prominence formed by the root of the incisor to the anterior termination of the masseteric ridge. The anterior third of the insertion is purely aponeurotic. The chief use of the masseter major is in closing the jaws, both while gnawing with the incisors and champing the cheek teeth; in addition,

it may be of some help in certain forward movements of the mandible, although this is not the case with many mammals, including man.

**TEMPORALIS.**—This muscle may also be divided into a superficial and a deep portion. The former (see fig. 5, *a*), well developed in the present animal, arises along the lambdoidal crest and thence forward along the temporal ridge to the posterior termination of the interorbital constriction. It then descends anteriorly and laterally, wholly within the orbit, to an insertion upon the anterior

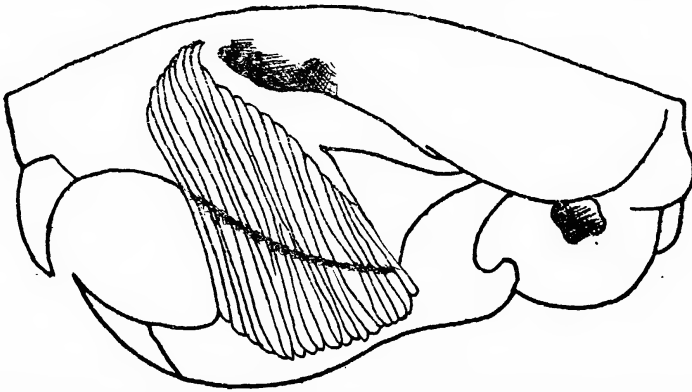


FIG. 6.—Superficial portion of the masseter major (masseter superficialis removed)

edge of the coronoid process, and thence anteroinferiorly to the junction of the latter process with the main body of the ramus. The anterior portion of the insertion is wholly by a thin, aponeurotic sheet. The deep part (see fig. 4, *a*) arises upon the postorbital process and inserts upon the medial side of the coronoid process from its tip to the temporal fossa of the mandible (between the molars and the coronoid process).

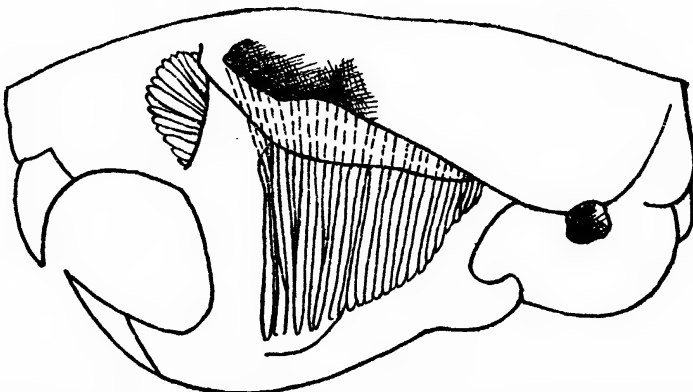


FIG. 7.—Head, showing the deep portion of masseter major, including the anterior portion of the ante-orbital branch (masseter superficialis, zygomatica, and the superficial part of major, removed)

The temporal is a very powerful muscle and is used not only for closing the mouth, but to some extent for drawing the mandible backward.

**PTERYGOIDEUS EXTERNUS.**—This muscle arises from the lateral side of that portion of the external pterygoid plate anterior to the foramen rotundum, and inserts upon the inferior border of the medial process on the head of the condyle.

**PTERYGOIDEUS INTERNUS.**—This arises from the pterygoid fossa and inserts upon the medial surface of the angular process of the mandible. Both this muscle and the pterygoideus externus are used in lateral movements of the jaw, such motion being produced by their alternate action first from one side then from the other.

## THE SKULL

It is necessary to have some criterion on which to base a consecutive arrangement of the skulls for the purpose of study. The most desirable, of course, would be the mean of a variety of characters showing age, such as amount of ridging, angularity, and general development of the skull; but it is impossible for the human eye to appreciate such fine gradations as are involved, and more definite methods must be used. Although some of the smaller skull dimensions might be employed with excellent results, it seems preferable to make use of one of the longer measurements. The total length of skull is perhaps usually favored above all others. In some mammals this measurement is an excellent one, but in others, whose nasals project sufficiently for these to constitute the anterior point of the measurement, it is open to the serious objection that the fragile tips of the nasals are very prone to injury; hence, not only may the skull be damaged to this extent, but the subsequent measurements will fluctuate with the amount of such damage.

The length of the hensel (distance from the inferior lip of the foramen magnum to the posterior margin of the alveolus of the incisor) is free from this objection, and may be an excellent measurement to use. As the precise relative position of the inferior lip of the foramen magnum is apt to vary, however, the writer prefers to employ the following measurement:

**CONDYLO-BASILAR LENGTH.**—This is the distance from the condyle to the posterior margin of the alveolus of the incisor upon the same side. It constitutes the criterion upon which the arrangement of the present series is based. As previously mentioned, it is the most natural arrangement, when considered with reference to weight and size of the animal and its pelage, that could be devised. The series of skulls thus arranged exhibits a minimum of individual variation.

The skull as a whole is characterized by no marked peculiarities, and its specific characters need not be discussed.

The skull is properly divisible into two parts—the cranium proper and the face. The former should be considered as being made up of three rings as follows: The posterior ring consists of the occipital. In the middle ring are the interparietal, parietals, basisphenoids and alisphenoids, and the temporals, including the squamosals, mastoids, and audital bullae. The anterior ring is formed of the frontals, orbitosphenoids, presphenoids, and the ethmoid.<sup>4</sup> After a fashion, each of these three rings forms an integral part and may be said to vary individually as independently as its articulations with adjoining rings will allow. The face is made up of the nasals, premaxillae, maxillae, jugals, lachrymals, palatines, turbinals,<sup>4</sup> and vomer.<sup>4</sup>

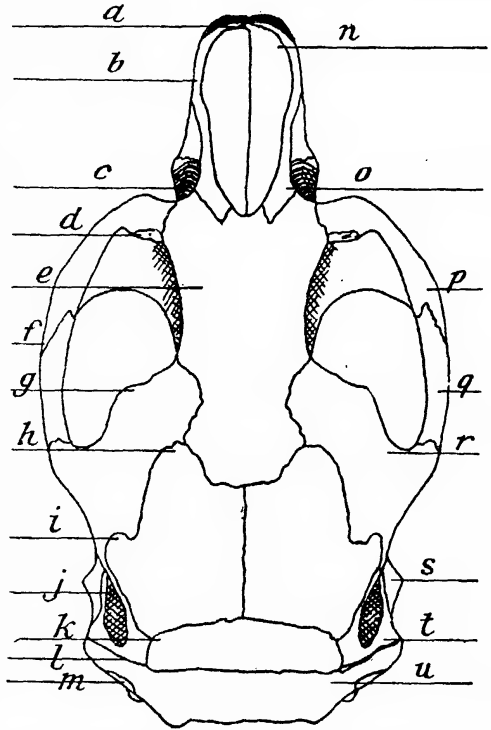


FIG. 8.—Diagrammatic dorsal aspect of skull of adult *Microtus m. yosemite*. a, Anterior nares; b, premaxilla; c, anteorbital fossa; d, lachrymal; e, frontal; f, zygomatic arch; g, postorbital process; h, anterior projection of parietal; i, lateral projection of parietal; j, prelambdoidal fenestrum; k, interparietal; l, lambdoidal crest; m, mastoid; n, nasal; o, ascending branch of premaxilla; p, maxillary root of zygoma; q, jugal; r, squamosal; s, audital bulla; t, squamosal; u, occipital

<sup>4</sup> These bones need not here be discussed, as the impossibility of examining them without severely damaging the skull renders them useless as characters in ordinary systematic mammalian studies.

In considering the variation of the skull with age, the anterior ring, chiefly the frontals, of the cranium is "dead center," from which there is development anteriorly and posteriorly. For the purpose of following this general development of the skull with age, there have been selected from the series of females the largest adult, a medium subadult, and the smallest juvenile. The general conclusions reached therefrom have been checked with the remainder of the series, but details regarding only these three will be presented in order to avoid a needless amount of tedious statistical matter.

Females were selected in preference to males, because their characters are in most respects more uniform and less extreme.

Skulls of juveniles are, of course, much weaker than are those of adults. All surfaces are rounded and the processes are more obscure and poorly defined. This is a natural correlation with the weaker, undeveloped condition of the muscle system. In addition, the antero-posterior dimension of the skulls of juveniles have the superficial appearance of stubbiness, with relatively much shorter rostrum and diastema, crowded condition of the pterygoid region, and smaller bullae; in transverse dimensions, wider interorbital, narrower zygomatic spread, and wider braincase. A critical study of these points shows that some of the differences enumerated above are merely optical illusions brought about by the lack of strength and ridging and the weak processes of the juvenile skull. In reality, the skulls of the two extremes in age are remarkably and surprisingly similar in relative measurements, as shown both by the figures and camera-lucida studies. In proportion of nasals to condylo-basilar length the three skulls of the

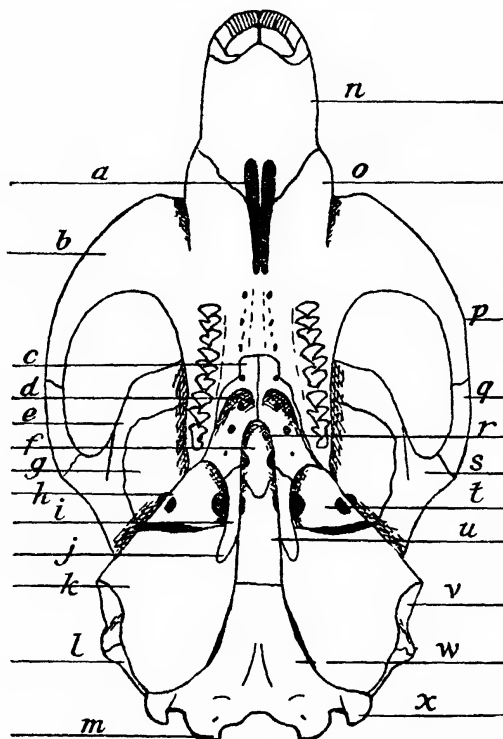


FIG. 9.—Diagrammatic ventral aspect of adult skull. a, Incisive foramen; b, zygomatic process of maxilla; c, palatal; d, palatal pit; e, squamosal; f, presphenoid; g, alisphenoid; h, external pterygoid plate; i, pterygoid plate; j, hamular process; k, auditory bulla; l, mastoid; m, condyle; n, premaxilla; o, maxilla; p, anterior angle of xygoma; q, jugal; r, interpterygoid fossa; s, glenoid fossa; t, pterygoid fossa; u, basisphenoid; v, external auditory meatus; w, basioccipital; x, paroccipital process.

ages selected indicate that the greatest difference between them is less than 2 per cent; condylo-zygomatic to condylo-basilar, 1.6 per cent; nasals to condylo-zygomatic, 2.7 per cent. The difference in the proportion of the zygomatic width to condylo-basilar length is but 0.6 per cent.

The capacity of the braincases in the whole series of males was investigated by filling them with No. 10 shot (see Table I, p. 981). The results are perhaps not so accurate as would be the case had mercury been employed, but the difficulty of stopping up all the vacuities and foramina of the cranium rendered the use of this metal impractical. The shot, however, proved satisfactory, and tests have shown that the normal error is only from 1 to 2 per cent. The average capacity of the braincase in the eight normal, adult males with greatest condylo-

basilar length is 115.2 pellets, while that of the eight smallest, normal subadults is 110.3, or 95.6 per cent. A similar comparison of the condylo-basilar length of these individuals gives 91.6 per cent. Comparison of the braincases of the largest superadult and smallest juvenile male results in figures that are misleading (73.8 per cent), for the reason that the brain capacity of the former is abnormally large. Comparison of the average of the three largest specimens with that of the three smallest, however, gives 83.4 per cent, while a similar treatment of their condylo-basilar lengths results in a percentage of 84.5. It is perhaps wiser to ignore the fact that the two latter percentages occur in reverse order to those first stated, for as the difference is small this is very likely due to individual variation. As this latter is considerable, the reader must judge for himself whether the figures indicate true conditions, and whether or not to consider the braincases of the younger animals proportionately larger than those of the older ones. At any rate, this difference with age is assuredly much less than has been popularly supposed. In skulls with the largest braincases the increased capacity is usually due to a slight proportional increase in the length, rather than in the breadth, of the cranium.

Aside from such facts as are offered above, the external surface of the braincase has certain details that do make it appear definitely of greater relative size in comparison with the whole skull in the case of the juvenile. While the con-

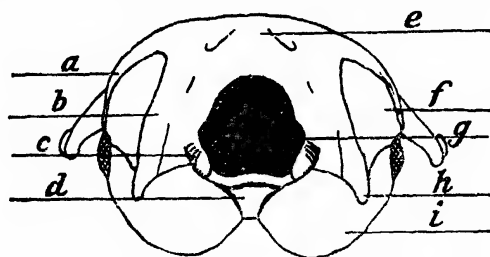


FIG. 11.—Diagrammatic posterior aspect of adult skull. *a*, Lambdoidal process; *b*, exoccipital; *c*, condyle; *d*, basioccipital; *e*, supraoccipital; *f*, mastoid; *g*, foramen magnum; *h*, paroccipital process; *i*, audital bulla.

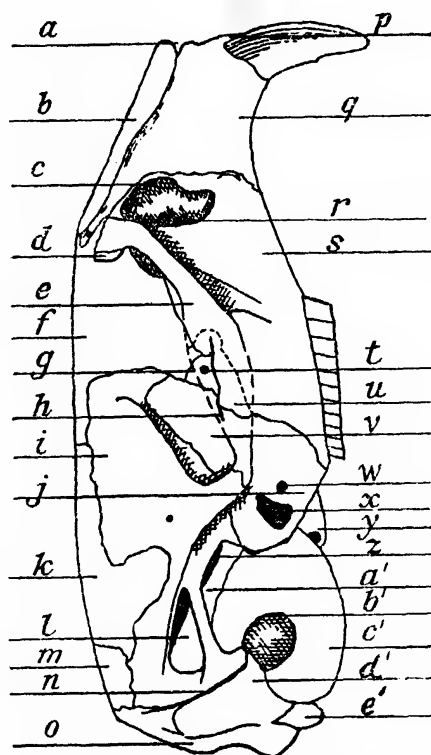


FIG. 10.—Diagrammatic lateral aspect of adult skull. *a*, Anterior nares; *b*, nasal; *c*, antorbital fossa; *d*, lachrymal; *e*, zygomatic process of maxilla; *f*, frontal; *g*, orbitosphenoid; *h*, sphenoidal fissure; *i*, squamosal; *j*, external pterygoid plate; *k*, parietal; *l*, prelambdoidal fenestrum; *m*, interparietal; *n*, lambdoidal crest; *o*, occipital; *p*, incisive process of premaxilla; *q*, premaxilla; *r*, antorbital foramen; *s*, maxilla; *t*, optic foramen; *u*, jugal; *v*, alisphenoid; *w*, foramen rotundum; *x*, foramen ovale; *y*, internal pterygoid plate; *z*, premastoid vacuity; *a'*, mastoid; *b'*, external auditory meatus; *c'*, audital bulla; *d'*, mastoid; *e'*, paroccipital process.

dylo-basilar length of the smallest female is 80.8 per cent that of the largest the distance between the prelambdoidal fenestrations of the squamosals is actually 0.4 mm. greater in the juvenile, and the transverse measurement taken between the posterior termination

of the postorbital processes is 92 per cent that of the adult. The proportional width (of braincase only) at the zygomatic processes of the squamosals is also about 92 per cent; but this is a very difficult measurement to take with accuracy

The interorbital width varies practically none at all with age, and as it is therefore proportionately so much greater in young examples, it adds much to the short appearance of the skull. Still another contributing cause is the weakness of the zygomatic processes of the maxillae in very young animals.

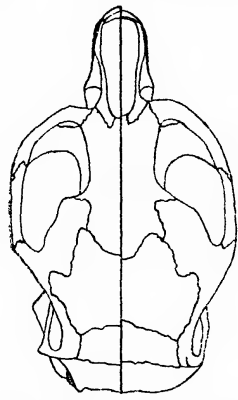


FIG. 12.—Variation of the skull with age in *Microtus m. yosemite*: dorsal aspect. Left half of the skull of adult No. 3835 upon the left, separated by medial line from right half of skull of juvenile No. 3857 upon the right; the condylo-basilar dimension of both magnified to the same size

Although the nasals are of practically the same relative length in the two extremes of age, the rostrum is slightly shorter in juveniles, the brevity of which is further accentuated by the lack at this age of pronounced anterior projection of the incisors. The transverse measurement of the superior portion of the rostrum between the anteorbital fossae may be actually just as great in juveniles, although this apparently is not always the case. Where such proportionately greater breadth exists it still further lends the appearance of shortness to the juvenile rostrum.

Inferiorly, the bullae are relatively slightly larger in juveniles, especially in the width of the anterior portion, but the pterygoid region is considerably shorter in the case of animals of this age. In the two smallest juveniles available the incisive foramina are located relatively farther forward than in adults, which probably means that the anterior growth of the premaxillae, as age advances, is at a greater rate than that of the maxillae.

TABLE III.—Measurements of the skulls of males (in millimeters)

Collection number	Condylo-basilar length	Condylo-zygomatic length	Alveolo-basilar length	Condylo-palatal length	Zygomatic width	Interorbital width	Lambdoidal width	Height	Tooth row	Incisive foramina	Alveolar width	Nasals	Angular process
3835	28.2	23.3	16.5	13.4	17.0	3.7	13.0	10.9	7.2	5.6	5.9	8.0	6.2
3837	27.7	23.0	16.3	13.0	17.3	3.8	12.9	11.2	7.0	6.0	5.7	8.8	5.8
3812	27.6	22.9	16.4	13.1	17.9	3.9	13.5	10.9	7.0	5.8	5.9	8.3	6.2
3826	27.5	22.9	16.2	13.3	16.7	3.9	12.9	11.2	7.0	5.6	5.9	8.0	6.3
3853	27.5	22.9	16.3	12.8	16.6	4.1	12.6	10.8	6.8	5.7	5.6	8.6	5.6
3825	27.3	22.9	16.0	13.3	16.8	4.0	12.9	10.8	6.7	5.8	5.7	8.3	5.8
3854	27.3	22.7	15.9	13.0	17.1	3.7	12.5	11.1	6.8	5.6	5.7	8.3	5.9
3822	27.2	22.5	16.1	12.9	16.6	3.7	12.7	10.9	6.9	5.4	5.7	7.7	5.9
3818	27.2	22.5	16.5	13.2	17.0	4.1	12.6	10.8	7.2	5.7	6.1	8.8	5.6
3864	27.1	22.5	16.0	12.8	16.8	3.8	12.9	11.2	6.8	5.8	5.7	8.2	5.5
3846	26.1	21.9	15.6	12.3	16.5	3.8	12.4	10.9	6.7	5.2	5.6	7.8	5.6
3855	25.9	21.6	15.3	12.3	16.2	3.8	12.0	10.8	6.8	5.6	5.7	7.6	5.1
3848	25.9	21.2	15.2	12.1	15.1	3.7	12.1	10.7	6.4	5.7	5.3	7.3	5.2
3862	25.7	21.3	14.8	12.1	15.3	3.6	12.3	10.2	6.7	5.0	5.7	7.5	5.6
3883	25.6	21.7	15.1	12.2	15.5	4.0	12.3	10.6	6.7	5.5	5.5	7.5	5.5
3820	25.6	21.3	15.0	12.2	14.9	3.6	11.7	10.2	6.5	5.5	5.4	7.3	5.2
3841	25.4	21.2	14.7	12.1	15.4	3.9	12.1	10.4	6.5	5.5	5.5	7.0	5.4
3870	25.1	20.9	14.8	11.2	15.6	4.0	12.2	10.8	6.6	5.2	5.5	7.3	5.6
3847	24.9	20.7	14.8	12.0	15.2	3.8	12.2	10.5	6.3	5.2	5.3	7.3	5.4
3867	24.7	20.9	14.6	12.3	14.7	3.9	11.9	10.7	6.5	5.3	5.4	6.9	5.5
3887	24.5	20.3	14.3	11.4	14.4	3.6	11.7	10.2	6.1	5.4	5.4	6.9	4.6
3814	24.4	20.5	14.3	11.4	15.0	3.7	11.9	10.2	6.2	5.2	5.4	7.0	4.7
3849	24.3	20.5	14.3	11.2	15.3	3.7	12.1	10.4	6.4	5.2	5.5	7.0	5.0
3801	23.4	19.8	14.2	11.2	14.1	3.5	11.4	9.7	6.2	4.9	5.3	7.0	4.8
3857	22.7	19.3	13.5	10.6	14.4	3.6	11.2	10.1	5.9	4.7	5.2	6.5	4.6
<sup>a</sup> Averages	26.2	21.8	15.45	12.45	16.0	3.84	12.4	10.7	6.68	5.5	5.6	7.74	5.6

<sup>a</sup> Average measurements of 19 (roughly, three-quarters) of the skulls of males, the largest two and smallest four specimens being eliminated from consideration.

TABLE IV.—Measurements of the skulls of females (in millimeters)

Collection number	Condyllo-basilar length	Condyllo-zygomatic length	Alveolo-basilar length	Condyllo-palatol length	Zygomatic width	Interorbital width	Lambdoidal width	Height	Tooth row	Incisive foramina	Alveolar width	Nasals	Angular process
3815.....	27.6	22.9	16.2	12.5	17.0	3.9	12.8	11.1	6.9	5.6	5.7	8.5	5.5
3856.....	27.4	22.8	16.2	13.0	16.5	3.9	12.8	11.2	6.9	5.5	5.8	8.3	5.2
3865.....	26.9	22.2	15.9	12.9	16.0	3.6	12.4	10.8	7.1	5.6	5.8	8.2	5.2
3819.....	26.9	22.2	16.5	12.3	17.1	3.7	12.7	10.9	7.2	5.5	5.9	8.3	5.5
3844.....	26.8	22.3	15.8	12.3	17.6	3.7	13.1	11.1	7.0	5.7	6.0	8.3	5.7
3871.....	26.8	22.1	15.3	13.1	15.9	3.7	12.4	10.7	6.7	5.5	5.7	7.6	6.1
3816.....	26.8	22.0	15.7	12.5	15.9	3.7	12.1	10.6	6.6	5.3	5.6	8.0	6.2
3845.....	26.8	22.3	16.1	12.0	16.6	3.8	12.6	10.9	7.0	5.3	5.8	8.3	5.6
3817.....	26.7	21.7	15.5	12.8	15.9	3.6	12.3	10.6	6.6	5.4	5.5	7.9	5.6
3884.....	26.7	22.0	16.2	12.3	16.4	4.1	12.8	11.0	7.3	5.3	5.6	8.0	5.2
3836.....	26.7	22.2	15.6	12.7	16.8	3.9	12.2	10.3	7.4	5.2	5.8	7.8	5.7
3881.....	26.4	21.7	15.9	12.1	16.5	3.9	12.3	10.9	6.9	5.5	5.8	7.7	5.5
3877.....	26.0	21.3	15.8	12.1	16.1	3.6	12.4	10.5	7.0	5.0	5.9	7.4	5.1
3863.....	25.8	21.5	16.0	12.1	16.8	4.0	12.3	11.0	7.2	5.5	5.7	7.7	5.5
3869.....	25.8	21.2	15.1	12.1	15.4	3.8	11.7	10.7	6.4	5.1	5.5	7.7	5.2
3876.....	25.7	21.2	15.3	12.2	16.6	3.7	12.3	10.6	6.6	5.5	5.7	7.5	5.6
3831.....	25.7	21.1	15.6	12.1	15.8	3.9	12.3	10.6	6.9	5.4	5.4	7.4	5.6
3821.....	25.6	21.4	15.2	11.8	15.4	3.9	11.9	10.6	6.5	5.5	5.4	7.4	5.0
3840.....	25.5	21.2	15.1	11.5	15.2	3.8	11.9	10.5	6.4	5.4	5.6	7.3	5.0
3880.....	25.5	21.4	15.2	12.5	15.5	4.0	12.0	11.1	6.6	5.5	5.6	7.5	5.4
3830.....	25.5	21.5	15.0	11.8	15.1	3.8	11.9	10.8	6.5	5.4	5.4	7.4	5.2
3827.....	25.4	21.7	14.6	12.1	15.9	4.0	12.2	11.0	6.6	5.2	5.5	7.2	5.3
3828.....	25.4	20.9	15.1	11.8	15.0	3.7	11.9	10.5	6.0	5.3	5.4	7.8	4.7
3872.....	25.4	21.2	15.0	12.2	15.1	3.7	11.8	10.8	7.4	5.3	5.6	7.5	5.4
3838.....	25.4	20.9	15.1	12.1	15.0	3.6	11.7	10.8	6.2	5.2	5.4	7.5	4.7
3850.....	25.2	20.8	14.8	11.7	14.6	3.7	11.6	10.4	6.7	5.3	5.6	7.1	4.7
3885.....	25.1	20.7	15.2	11.8	15.4	3.9	12.0	10.6	6.9	5.6	5.6	7.0	5.0
3886.....	25.0	20.7	14.6	11.3	15.1	3.8	11.4	10.9	6.1	5.3	5.6	7.0	5.1
3873.....	24.9	21.2	14.7	12.2	15.0	3.6	11.2	10.6	6.6	5.0	5.5	7.0	5.2
3813.....	24.9	21.0	14.8	11.9	14.6	3.6	11.5	10.1	6.8	5.8	5.4	7.4	4.7
3882.....	24.9	21.2	14.9	11.5	14.8	3.8	11.9	10.5	6.7	5.2	5.4	7.1	4.8
3839.....	24.9	20.7	14.7	11.3	14.3	3.6	11.4	10.3	6.4	5.4	5.4	7.1	4.7
3852.....	24.8	20.6	14.7	11.2	15.3	3.8	11.7	10.3	6.4	5.2	5.5	7.3	5.0
3829.....	24.7	20.5	14.9	11.5	14.7	3.7	11.6	10.2	6.5	5.4	5.4	7.4	4.7
3842.....	24.6	20.5	14.7	11.4	14.8	3.6	11.4	10.5	6.3	5.1	5.4	6.9	4.9
3861.....	24.6	20.3	14.6	11.5	15.0	3.6	11.4	10.5	6.4	5.0	5.4	7.2	5.0
3866.....	24.6	20.3	14.8	11.3	15.2	3.6	11.4	10.6	6.5	5.5	5.3	6.8	5.1
3851.....	24.5	20.6	14.9	11.7	15.4	3.8	12.0	10.5	6.8	5.1	5.3	7.1	5.0
3888.....	23.8	19.8	14.5	11.2	14.3	3.7	11.2	10.8	6.6	5.0	5.3	7.1	4.8
3832.....	23.2	19.5	14.1	11.0	14.0	3.6	11.2	10.0	6.3	4.5	5.2	6.9	4.7
3833.....	22.3	18.8	13.3	10.2	13.6	3.8	11.2	9.8	6.0	4.1	5.0	6.5	4.5
α Averages.....	25.7	21.34	15.27	12.0	15.63	3.77	11.99	10.65	6.7	5.35	5.5	7.5	5.24

α Average measurements of 32 (roughly three-quarters) of the skulls of females, the three largest and six smallest specimens having been eliminated.

In lateral profile the skulls of juveniles are evenly rounded, with a sharper curve barely perceptible at the posterior portion of the frontals and no undue prominence at the anterior margin of these bones. In older animals the dorsal profile is very much less rounded. In the larger subadults the greatest prominence has shifted to the vicinity of the nasal-frontal suture, while in adults this has increased, with considerable angularity and pronounced declivity of the rostrum in many cases. There is individual variation of limited extent in this outline and in the exact age at which the change is apparent. In transverse outline from a posterior view the braincase in juveniles is proportionately higher and therefore narrower, and more evenly arched, or in other words, more nearly circular; while in the case of adults this outline is much more oval.

#### COMPOSITE MEASUREMENTS

The composite measurements have been as follows:

CONDYLO-BASILAR LENGTH.—The average of this measurement for the 19 intermediate males (see footnote, Table III, p. 996) is 26.2 mm., with extremes



of 27.6 and 24.5, a difference of 3.1 mm., or 11.8 per cent of the average. For females the average is 25.7 mm., with extremes of 26.9 and 24.6, a difference of 2.3 mm., or only 8.9 per cent. These figures, as well as subsequent ones, are a further indication of the fact that males exhibit greater extremes of development with less conservatism of characters than do females.

**CONDYLO-ZYGOMATIC LENGTH.**—From the condyle to the antero-superior edge of the zygomatic process of the maxilla. The tables give an average of 21.8 for males, with extremes of 22.9 and 20.3, a difference of 2.6 mm., or 11.9 per cent of the average. The average for females is 21.34, with extremes of 22.3 and 20.5, a difference of 1.8 mm., or 8.4 per cent. This is an excellent measurement, dependable, with little individual variation, and is correlated closely with condylo-basilar length. The latter measurement, however, is preferable, and in cases where it is taken, as it should be, it is of value when used with the condylo-basilar measurement as an index to the development of the rostrum.

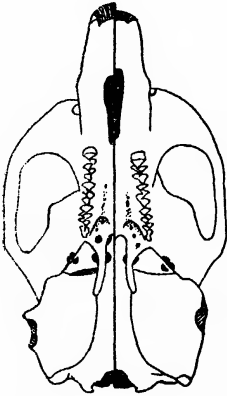


FIG. 13.—Variation of the skull with age in *Microtus m. yosemitae*: ventral aspect. Left half of the skull of adult No. 3855, upon the left, separated by medial line from right half of skull of juvenal No. 3857, upon the right: condylo-basilar dimensions of both magnified to the same size

**ALVEOLO-BASILAR LENGTH.**—The distance from the posterior end of the last molar to the posterior edge of the incisive alveolus. For males the average of this measurement is 15.45 mm., with extremes of 16.5 and 14.3, a difference of 2.2 mm., or 14.2 per cent of the average. For females the average is 15.27 mm., with extremes of 16.5 and 14.6, a difference of 1.9 mm., or 12.4 per cent. This is a rather good measurement, as it seems to be of considerable importance in general diagnostic work among microtines. Its variation is not excessive with age nor individually, and it is preferable to the measurement of the diastema, to which it is somewhat similar, for the latter must be measured with greater care in order to prove accurate.

**CONDYLO-PALATAL LENGTH.**—This measurement is from the condyle to the anterior point of the bony shelf of the palate. The average for this measurement in males is 12.45 mm., with extremes of 13.3 and 11.2, a difference of 2.1 mm., or 16.8 per cent. For females the average is 12 mm., with extremes of 13.1 and 11.2, a difference of 1.9 mm., or 15.8 per cent. This measurement varies to a considerable extent individually and more with age, but it is probably the most valuable measurement to use in precise work as an index to the development of this portion of the skull. The chief and probably the only objection to its use is the fact that while taking it the delicate processes often present upon the bony shelf of the palate, which may be of the greatest importance in specific or group diagnoses, are extremely subject to injury.

**HEIGHT OF SKULL.**—The perpendicular distance from a plane passing from the most inferior point of the bulla along the crown of the most prominent molar to the highest point of the cranium. The average of this measurement for males is 10.72 mm., with extremes of 11.2 and 10.2, a difference of 1 mm., or 9.3 per cent. For females the average is 10.65 mm., with extremes of 11.1 and 10.1, again a difference of 1 mm., or 9.4 per cent. The height of the cranium is an extremely useful measurement to take, especially when it is used in comparative work with the condylo-basilar length as an index. Another character that is valuable in the case of microtines is the angle formed by the molar row with the plane passing from the bullae to the most prominent molars. In many

other families and subfamilies, however, height of the cranium can not satisfactorily be taken in just this manner, but special methods must be devised, as height from the pterygoid plates, or measured from the alveolar border between the penultimate and last molars. A scrutiny of the series at hand shows that the actual height of the posterior portion of the braincase differs hardly at all

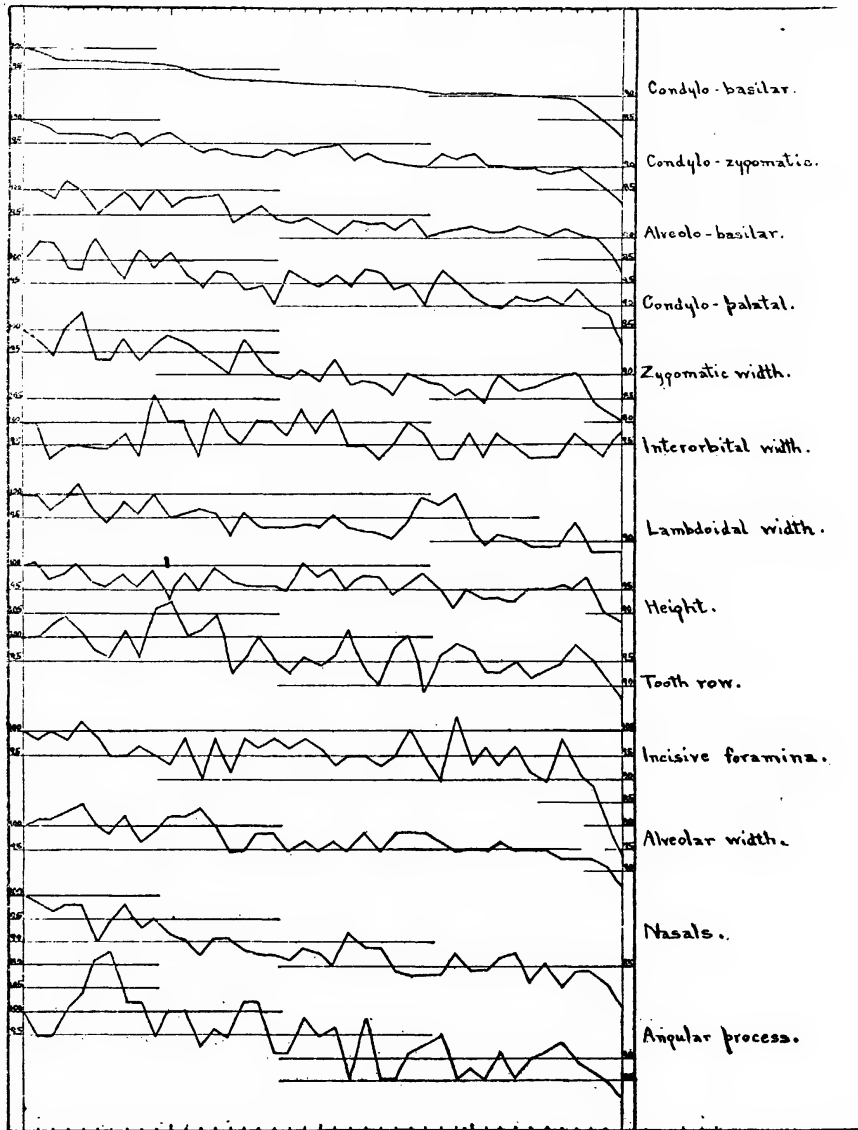


FIG. 14.—Graph for skull measurements of the female series, reduced to percentages, with the largest skull considered as par, and arranged according to condyle-basilar sequence

with age, although there is some individual variation in this measurement. The difference with age shown in the tables is caused chiefly by variation in the infero-superior dimension of the bullae, but the height measured from the inferior border of the foramen magnum is almost uniform. Unfortunately, without calipers of special design it is practically impossible to measure the height of skull after this fashion with a satisfactory degree of uniformity.

**ZYGOMATIC WIDTH.**—The greatest spread of the zygomatic arches. The average of this measurement for males is 16 mm., with extremes of 17.9 and 14.4, giving a variation of 3.5 mm., or 21.9 per cent. For females the average is 15.63 mm., with extremes of 17.6 and 14.6, a difference of 3 mm., or 19.2 per cent.

As can be seen, the variation, both individual and with age, of this measurement is great, and the variability in the exact configuration of the zygomata is even greater.

The development of the zygomatic region is considerably involved, and this, together with the temporal and lambdoidal ridges, reflects the influences of the muscular growth more decidedly than any other portion of the skull. Excessive development at these points is a result almost if not quite entirely of a similar excessive development of the major muscles of the head. The latter state of affairs is, in turn, brought about largely by the character of the food and feeding

habits of the individual, although, as previously mentioned, there are probably inherent trends, evolutionary, specific, etc., partially influencing the muscular development and complicating the results.

A digression here may be permitted to indicate that it may occur to the reader that if the above statement be true a captive animal that was fed exclusively on pap of some sort would grow to full size still having a skull that exhibited all the weakness of the juvenile cranium. Such would not necessarily be the case, however, for other investigations have indicated that the unworn molar pattern

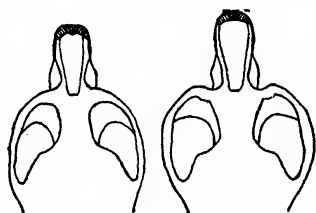


FIG. 15.—Individual variation in the lateral development of the zygomatic arches of adult mice. No. 3848 on left; No. 3864 on right

of nestling meadow mice is worn down a surprising amount during a space of two days, apparently merely by a gritting together of the teeth. Hence, an individual, even though fed on soft food, might be stimulated thereby to an unusual amount of teeth-gritting, gnawing wood, or even a piece of loose wire, as the writer has seen captive specimens do by the half hour at a time, apparently for the sole purpose of exercising the jaws and the muscles. The inherent desires of such an individual, therefore, might nullify the effects of any deficiency in toughness of the diet.

Scrutiny of the series shows that there is only slight correlation (aside from ridging) between the cranium proper and the anterior half of the skull. In other words, the squarest, most angular skull with greatest spread of zygomata may have a cranium no larger than examples showing marked weakness in these respects. In addition, although skulls with great zygomatic width are usually the most heavily ridged and angular, these are not necessarily the largest skulls, and there is considerable variation in this respect, as mentioned in the last paragraph. The distance between the glenoid cavities varies practically not at all individually, showing that there is corresponding uniformity in the distance between the condyles of the mandible. There is much variation in the degree of lateral projection of the zygomatic processes of the squamosals, this conforming to the amount of angularity exhibited by the remainder of the zygomata. It is in the region of the zygomatic processes of the maxillae, from which arise the masseter muscles, that any difference in the strength and angularity of the skull is most pronounced, for it is there that variation in the development of this great muscle is directly manifested.

In specimens showing the greatest angularity and width of the zygomata the septum formed by the maxillary root is correspondingly extensive, the antero-superior indentation more accentuated, and the superior border often heavier. As the process extends from the long axis of the skull at more nearly a right angle, the anterior angle of the zygomatic arch is better defined in the examples with greatest zygomatic width. Correlated with the above points is corresponding angularity of the lower jaw, as discussed under the proper heading.

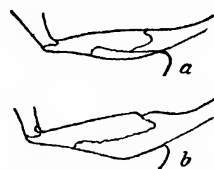


FIG. 16.—Individual variation in depth of the zygomatic arch of adults. Right lateral aspects of No. 3835, a; No. 3845, b

Measurements of height of the rostrum are not here presented for the reason that it is impossible to obtain them with any sort of uniformity. This, nevertheless, is a character of great importance in specific diagnosis. Individual variation is great, especially in the height of the posterior portion of the rostrum. Maximum height, or depth, of this member is practically always accompanied by pronounced declivity of the supero-lateral profile. In other words, great depth of the skull is accentuated in this region and but little or not at all in the anterior portion of the rostrum. There is equal variation in its ventral profile; in some this is almost straight, while in others, chiefly those with considerable depth of this member, it exhibits pronounced concave curvature. Although as a rule there is no great difference in the relative length of the rostrum, several individuals have rostra that are conspicuously stubby, and this is correlated only partially with length of the nasals. Female No. 3827 is especially noteworthy in this respect, as an examination of its condylo-basilar, condylo-zygomatic, and nasal measurements, in comparison with those of No. 3876, for instance, will show.

#### BONES OF THE SKULL

**OCCIPITAL.**—This bone is divided into three areas, the supraoccipital above, the exoccipitals laterally, and the basioccipital below. Superiorly the supraoccipital articulates with the interparietal and squamosals; the exoccipitals with the mastoids; and the basioccipital, skirting the auditory bullae, anteriorly with the basisphenoid. To the occipital bone are attached many of the powerful cervical muscles, and its configuration therefore reflects the degree of their development. A pronounced tilting forward of the occipital is acknowledged to be a modification brought about by fossorial habits and is supposed to have been caused by the stresses brought upon the bone and its attached muscles by the animal pushing aside the earth of the burrows with its head. Incidentally, the writer is not at all convinced that other life habits besides fossorial ones do not bring about somewhat similar results. At any rate, within the present series there is excessive variation in the forward inclination and all other aspects of the occipital when viewed vertically from above. In fact, hardly any two examples are precisely alike in the shape of the superior border and the irregularities of the posterior surface of this bone. These variations are present irrespective of age and are purely individual, the result of variation in the cervical musculature. There is similar variation in the visibility of the condyles when the skull is viewed from above; in some specimens they can not be seen, in a few they are prominent, but in the majority they are barely perceptible. This difference, largely individual, is usually caused by the configuration of the supraoccipital, but is also due to the fact that in the younger animals the condyles are shorter. There is like difference in the exact shape and size of the foramen magnum, irrespective of age.

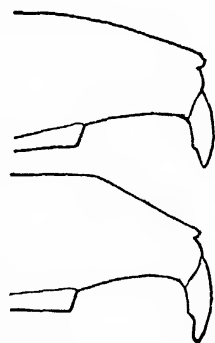


FIG. 17.—Individual variation in depth of rostrum and declivity of its dorsal outline in adult skulls. No. 3865 above; No. 3826 below

The superior borders of the lambdoidal processes of the occipital form the posterior faces of the lambdoidal crests. The degree to which these are developed depends upon the strength of the temporal muscles which are attached to them, and therefore their prominence is also correlated with the temporal ridges and to only a slightly lesser degree with the angularity and strength of the whole skull. There are lambdoidal crests of respectable size, however, in

animals that are barely matured, and upon which there is not as yet pronounced temporal ridging, and the variation is never so decided and abrupt as in the latter character. In juveniles the lambdoidal crests are inconspicuous.

There is some slight variation of the exoccipitals, and in young animals the sutures between these and the mastoids are relatively unraised. There is much variation, however, in the development of the paroccipital processes. In all but the smallest juveniles there is little age variation, but in these, although the processes are of respectable length, they closely embrace the bullae and are consequently directed slightly forward of the perpendicular. In adults and subadults they are, as a rule, only slightly longer proportionately, but the distal extremities are usually free from the bullae and are directed slightly rearward of the perpendicular. The variation is chiefly in the length and the degree of the infero-medial curvature of the tips, which in most of the specimens is moderate. The sole function of these processes seems to be for the attachment of the digastric muscles. This being the case, it appears that these processes are somewhat better developed in juveniles than the condition of the general musculature of the head at this age might lead us to expect, pointing to the probability that the digastric may be of more than ordinary importance in the economy of these mammals. In juveniles the margins of the basioccipital closely embrace the audital bullae, but in older examples there are vacuities at the lateral margins, these depending upon variation in the width of a slight constriction of the bone directly posterior to the basioccipital-sphenoid suture.

**INTERPARIETAL.**—This bone articulates anteriorly with the parietals, laterally to a varying extent with the squamosals, and posteriorly with the supraoccipital. It is highly variable in shape not because of any muscular attachments, but for the reason that its position renders it particularly subject to distortion by the development of adjoining parts of the cranium. The antero-posterior dimension varies from about 3.1 to 3.7 mm., and the transverse one from 6.1 in a very old individual to 8 mm. In the majority of individuals this bone is almost exactly twice as long as wide. There seems to be very little variation from juvenility to middle life. In the largest adults, however, the relative, and even actual, decrease in the prelambdoidal width of the braincase, with consequent narrowing of the distance between the temporal ridges, makes this bone *appear* narrower. In addition, the fact that it is narrower in some of these examples can not be ignored. This state of affairs may be attributable to strains imposed upon it by a greater growth force in the development and encroachment of the squamosals, with consequent absorption of the lateral margins of the interparietal, an explanation that is entirely logical if this really be the weaker bone, as seems to be the case.

Variation in the precise form of the anterior outline of the interparietal is almost infinite. In a few specimens this follows the form of a gentle, convex arc. In many others an almost straight line is broken by a rather sharp projection of the medial portion into a point. In the majority the sutures with the parietals follow a very wide obtuse angle, while in a number of others this angle is somewhat sharper and less obtuse. There is such variation, however, that it is not practicable to divide the series into definite groups based upon this character.

The suture with the squamosal may form almost a right angle with that with the occipital, but in the majority of cases the angle is of about 30° to 35° (the apex directed laterally). In still others the angle is considerably sharper, due to a greater prolongation of its apex. (See lower detail, fig. 18.) In almost all cases this suture pursues a very erratic course. That with the supraoccipital is usually practically straight or very gently curved. A slight, very obtuse

angle is sometimes formed, however, or an illy defined double curve. There is no definite variation with age.

**PARIETALS.**—Anteriorly this pair of bones articulates with the frontals, laterally with the squamosals, and posteriorly with the interparietal. Antero-medially there is a recession of the parietals, filled by a part of the frontals, and each has an anterior projection and a lateral one. These projections and recessions are least developed in juveniles, but after the attainment of sexual maturity there is negligible variation with age, though much individually. This is more pronounced in the anterior than in the posterior projection, though there are a few exceptions to this rule.

The temporal ridges are continuations, almost at right angles, of the lambdoidal crests. From the latter they cross the extreme lateral borders of the interparietal, extend across the bases of the lateral projections of the parietals, and almost always follow the borders of the anterior ones to their antero-medial tips, thence joining the interorbital ridging. Occasionally, however, the temporal ridges cut across a part of the anterior projections. Differences in their precise positions depend almost altogether upon similar differences in the shape of the parietals. The degree to which these ridges are developed is dependent upon the development of the temporal muscles. Therefore the strongest, most angular skulls have the best-defined temporal ridges, while in weaker, rounded skulls with narrow zygomatic width they are poorly defined. They may be lacking or quite distinct in medium-sized subadults, but in individuals which are fully adult they are always apparent, though in varying degree. On the whole, this character is perhaps better defined in males, although there are female skulls that are fully as heavily ridged.

**SQUAMOSALS.**—Each squamosal articulates posteriorly with the occipital and mastoid, medially to a slight extent with the interparietal as well as with the parietal, anteriorly with the frontal, and laterally with the alisphenoid and mastoid. The jugal articulates with the zygomatic root of the squamosal. Perhaps the chief variation in the outline of this bone lies contiguous to the

anterior projection of the parietal. In some individuals, especially the youngest ones, the squamoso-frontal suture begins at a point upon the antero-lateral margin of this projection, while in the majority it is situated relatively more medial. It is also influenced by the size of the anterior projection of the parietal. The squamosal-interparietal suture differs individually, but not with age, in extent and exact shape as pointed out in the discussion of the interparietal. The posterior border of the squamosal constitutes the anterior face of the lambdoidal crest and immediately anterior to this is situated the prelambdoidal fenestrum of the squamosal. This is not a true foramen in the bone, but rather seems to have been formed in remote times by an invagination of the bone from its posterior border, the posterior edges of the fenestrum having joined at a later period, the resulting false suture being plainly distinguishable in juveniles but disappearing with age in this species, although it is not so lost in some other genera of this subfamily. The exact size of this fenestrum is highly variable individually, in length

about 20 per cent. Its infero-lateral border is formed of a slender bar of bone, and infero-laterally to this is the premastoid vacuity, usually well defined but occasionally practically obliterated.

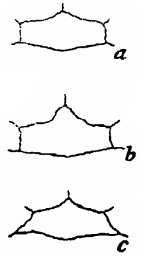


FIG. 18.—Individual variation of the interparietal in the skulls of adults. *a*, No. 3817; *b*, No. 3815; *c*, No. 3836

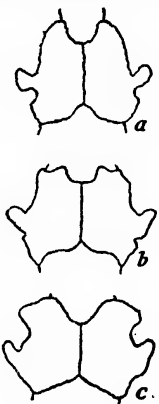


FIG. 19.—Individual variation of the parietals in adult skulls. *a*, No. 3815; *b*, No. 3863; *c*, No. 3864

There is much variation in the precise angle and the amount of projection of the zygomatic root of this bone. The greatest degree is, of course, met with in the case of individuals with maximum zygomatic width, and in these the angle is less acute. As previously mentioned, the zygomatic width is greatest in skulls that are most heavily ridged, and in such the postorbital processes of the squamosals are also somewhat better developed and more angular, the prominence of the latter depending directly upon the development of the deep portion of the temporal muscle. In animals with greatest development of this character the lateral slope of the anterior border of the braincase is most abrupt and squarish. In the case of heavily ridged and angular skulls the pull exerted by the powerful superficial part of the temporal muscles from their attachments upon the temporal ridges has actually flattened out the underlying portion of the squamosals between the ridges and the zygomatic processes, making them flatter than is the case in weaker skulls. Thus the temporal ridges, instead of being true ridges such as often occur in the interorbital region, are in reality the apices of angles formed by the superior and lateral surfaces of the cranium.

**MASTOIDS.**—The mastoid is very irregular in outline and closely embraces the audital bulla supero-medially. In the articulated skull it occupies the space on either side between the lambdoidal process, being bounded supero-medially by the exoccipital. The anterior portion, embracing the audital bulla, is visible within the prelambdoidal fenestrum and along the premastoid vacuity. The visible portion is quite variable, depending directly, upon analogous differences in the adjoining border of the occipital.

**AUDITAL BULLAE.**—Each one of these globular bones seems to form a true suture only with the mastoid, although there are false sutures connecting it more or less securely with the basi- and alisphenoids, squamosal, and basioccipital. Variation in the audital bulla is not of such a nature as to be measured readily, and one should be cautious in making statements regarding variations of an intangible sort. There is slight variation with age, the greatest being in the width of the anterior portion, and the whole being perhaps more globular in juveniles. There is little individual, proportionate variation, although a few specimens do have bullae that are slightly more globular than the average. It is in the relative size, or mass, that the chief difference lies. Upon the whole, this is of a sufficient amount so that it must be taken into consideration, but is not excessive. The auditory meatus varies but slightly in shape and size. In adult skulls, however, the antero-superior border projects laterally to a considerably greater extent than is the case with juveniles.

**SPHENOIDS.**—The articulations between the sphenoid bones are not always to be distinguished without difficulty in skulls of such small size. The alisphenoid articulates with the squamosal, frontal, maxilla, and the other sphenoids. Superiorly the suture formed by its connection with the squamosal extends forward from a point barely inferior to the premastoid vacuity to its junction with the frontal at a point slightly antero-medial to the postorbital process; thence it descends along the frontal to the orbitosphenoid and the sphenoidal fissure. From the latter the maxillary-alisphenoid suture extends obliquely rearward to join the external pterygoid plate immediately posterior to the third molar. The alisphenoid constitutes the entire lateral surface of this plate and the posterior portion of its medial surface, as well as the posterior portion of the floor of the pterygoid fossa. Although there are, of course, many differences in the precise configuration of this bone, these are relatively slight in degree. The most apparent difference occurs in animals of the two extremes of age through the lengthening of the pterygoid region as the animal grows, with consequent

elongation of the external pterygoids to form an angle with the axis of the skull of increasing acuteness, this in response to the development of the pterygoid muscles, chiefly the internal ones. It should be noted that there occurs a minimum of variation in the size and depth of the pterygoid fossae, except with age as noted.

Between the maxilla, orbitosphenoids, and alisphenoids occurs the large sphenoidal fissure, through which pass several important nerves and blood vessels. Directly supero-lateral to the external pterygoid plate are situated the foramen rotundum and foramen ovale, the latter being the posterior one and by far the larger. In this, however, as in many other species, the foramen rotundum varies much in size, and in a few cases is virtually blended with its neighbor.

**BASISPHENOID.**—This bone articulates posteriorly with the basioccipital and anteriorly with the presphenoid. It then descends antero-inferiorly, articulating with the palatines to form all but the anterior base of the internal pterygoid plates. The numerous foramina and vacuities in the pterygoid region are large and extensive in the present species. Especially worthy of note is the



FIG. 20.—Individual variation in the palatal shelf and internal pterygoid plates of the adult skull. *a*, No. 3812; *b*, No. 3845; *c*, No. 3837; *d*, No. 3820

vacuity in the anterior portion of either internal pterygoid plate and those on either side of the presphenoid. At times these will be found to be of aid in diagnosis. On the whole, these vacuities are rather constant individually, but they are proportionately very much smaller in younger animals. The internal pterygoid plates not only appear to be relatively heavier in juveniles because shorter, but they are actually heavier (wider) in inferior aspect than in the majority of older examples. There is also considerable individual variation not only in the apparent thickness of these plates, but in the shape. In some cases the two are practically parallel, in the majority they diverge slightly, while in the case of a few there is a marked tendency for the posterior halves to flare.

**PRESPHENOID.**—In the present species, as in many, if not most, microtines, the juncture of the basisphenoids and presphenoids can not be distinguished with certainty, even in juveniles. It can be described in the genus *Ondatra*, however, and from analogy with that it is possible to state with a degree of certainty that this bone begins at a point slightly posterior to the posterior vacuities upon the floor (or rather roof) of the interpterygoid fossa. It thence extends anteriorly, in the form of a rod, to join the vomer. The slender bridges of bone joining it upon either side are the presphenoid processes of the basisphenoid.

**ORBITOSPHEOIDS.**—Externally the orbitosphenoid is of little consequence in the present species. It appears as a very small bone forming the antero-medial wall of the sphenoidal fissure and is pierced by the optic foramen. It articulates with the alisphenoid, maxilla, and frontal.

**PALATALS.**—The suture between the paired palatals can be distinguished only in the youngest juveniles at hand. Each of these bones constitutes the extreme anterior portion of the internal pterygoid plate, the anterior part of the pterygoid fossa, the anterior half of the medial face of the external pterygoid plate, and together, the palatal shelf. The palato-maxillary suture lies parallel and just medial to the molar alveoli, and the anterior border of the bone is situated at a point lying between the middles of the second molars. Important details to be considered are the shelf of the palate and the palatal pits. There is much variation in the width and shape of the former, this being of a nature impossible



to classify with precision. In the majority of individuals the shelf is squarish in general outline, there being a slight medial notch flanked upon either side by a minute process, often spinous. These processes, however, are sometimes lacking, and the notch varies in size, at times being entirely absent, so that the shelf is then practically unbroken in outline. In a few examples the entire anterior portion of the interpterygoid fossa gradually tapers to a point. This variation is not dependent upon age but is entirely individual. The lateral shelves of the palatines (antero-lateral to the fossa) are also quite variable in exact configuration, probably being largely influenced by the development of the internal pterygoid muscles. The depth of the palatal pits also varies individually to a great extent, though not relatively with age. Finally, the entire palatal bone is pierced by many minute foramina and studded with tiny pits, no two being precisely alike in this respect. Hence, it is seen that individual variation in the palatal region is so great and of so intangible a nature (the several types being so inextricably blended) that these characters can be used only after careful deliberation.

**FRONTALS.**—The suture between these paired bones is clearly distinguishable in juveniles, but has disappeared by the time that the animal has attained sexual maturity. Each frontal articulates with the parietal, squamosal, alisphenoid, maxilla, premaxilla, lachrymal, and nasal. The posterior portion is characterized by great individual variation caused chiefly by the position of the squamoso-frontal suture. The posterior point of this may be either lateral or medial to the margin of the interorbital constriction; or in other words, it may be situated at either the lateral or medial edge of the anterior projection of the parietal. Most of the other sutures vary but slightly, and there are no age differences save that in juveniles the irregularities of the bone are more poorly defined and less abrupt. There are some individual differences anteriorly, but these are discussed elsewhere.

The chief interest in this pair of bones lies in the interorbital region. The only variation in the width of this, measured at its narrowest portion, is individual. Not only is it actually just as great in young animals, but may be greater, while among the examples with narrowest interorbital are some of the largest adults. In normal males this averages 3.84 mm., with extremes of 4.1 and 3.6, a difference of 0.5 mm., or 13 per cent. In females, the average is 3.77 mm., also with extremes of 4.1 and 3.6, a difference of 13.3 per cent of the average.

Normally there are two interorbital ridges, these being in reality anterior extensions of the temporal ridges and terminating at the postero-lateral edges of the nasals. They are absent in the smallest juveniles, though definable in large juveniles and practically all older individuals. In a very few, however, they are to all intents absent. There is great variation in the degree of definition in these ridges, as well as in the distance between them and all other details. There is a tendency for them to coalesce with advancing age, with consequent elimination of the intervening sulcus. Most, but not all, of the largest adults thus have single interorbital crests. Similarly, the sulcus is quite wide in many of the smaller subadults, though narrower in the majority, while it is well-nigh obliterated in a few, even of those that are barely sexually mature. The writer is led to believe that in those large adults now having a sulcus the latter was of unusual width in their youth, and vice versa. Animals with a tendency toward coalescence of the ridges usually show indications of a strong, **angular** braincase. But, on the other hand, this is not the case in the individuals of such a large minority that one hesitates at reaching any definite decision as to cause. Males are somewhat more heavily ridged in this region than are females.

The anterior portion of the braincase, and therefore the posterior portion of the interorbital, is amenable to the effects of the anterior part of the temporal muscles. No important muscles of mastication arise from the anterior portion of the interorbital, such as would logically develop a crest or ridges, and other causes are therefore responsible for their formation. Explanatory theories may be suggested for the growth of such, but these can not be proved.

**LACHRYMALS.**—It was not discovered until after all skulls had been cleaned that in their preparation by means of a fine stream of water, the lachrymals had become loosened and washed away from all but a very few. This bone, however, evidently varies to only a slight extent. When the skull is viewed from above, the lachrymal is seen as a small bone upon the antero-supero-medial border of the orbit. It embraces a small portion of the frontal and then extends along the zygomatic root of the maxilla to slightly lateral of the small lachrymal process of this bone. There is also a thin plate extending anteriorly along the supero-medial face of the anteorbital foramen quite to the anterior opening.

**JUGALS.**—The jugal or malar articulates posteriorly with the corresponding process of the maxilla. There is some variation in this bone, such depending upon differences in the zygoma as a whole. The posterior suture is strong, the jugal fitting into an indentation in the process of the squamosal, dovetail fashion. The anterior suture is discussed under the next heading.

**MAXILLAE.**—Each of the two maxillae articulates with the frontal, premaxilla, lachrymal, jugal, orbitosphenoid, alisphenoid, and the palatine. The suture with the frontal occurs at the superior base of the zygomatic process, this uniting with the maxillo-premaxillary suture, which descends to join the incisive foramen at a point slightly anterior to its center. The junction between the two maxillae, extending from these foramina posteriorly to the palatines, is obliterated, even in the youngest animals at hand. Skirting the lateral edge of the palatine, the entire maxillary molar series on either side is included within the maxilla, which then extends from just posterior to the third molar antero-superiorly to the sphenoidal fissure. Thence the frontal-maxillary suture ascends anteriorly to about the center of the anteorbital foramen.

The suture with the frontal at the superior base of the zygomatic process is usually practically parallel with the axis of the skull, but it may slope antero-medially at a considerable angle in some cases. There is practically no variation in the remaining sutures. As previously indicated, there is great variation in the development of the zygomatic process of the maxilla. In juveniles this is weak, and it may be almost as lacking in strength proportionately in sub-adults. Robustness in this detail is correlated with strength in other portions of the skull and is the result of stimulation by masseter muscles that are stronger than the average. There is little difference, save in the degree of lateral spread, between an angular and a weak skull when viewed from in front. When examined from above, the zygomatic processes of the former will be seen to project from the skull more nearly at right angles. The slight indentation in the bone immediately antero-superior to the anteorbital foramen is always well defined in angular skulls, which may or may not be the case in weaker ones. The slight, spinous process upon the supero-postero-medial border of the root—the lachrymal process—is even more irregular, its precise development, or absence, depending upon the adjoining lachrymal. The exact position of the anteriormost point of the maxillary-jugal suture is quite variable. In some specimens it is situated about even with the anterior face of the first molar, but in the majority it is about one millimeter, and in a few a trifle more to the rearward. The jugal is slightly dovetailed into the maxilla, and the suture extends posteriorly

to within a couple of millimeters of the posterior angle of the zygoma. There is great variation in the supero-inferior aspect of the portion of the maxilla immediately anterior to the jugal. In some (as the largest male) this detail is shallow, while in others (as female No. 3845) it is nearly 50 per cent deeper (see fig. 16), due to the formation of a sharp sort of superior crest. This extreme development is not necessarily present in the most angular skulls, but may develop independently, and is probably in response to certain special stimulation by the deep part of the masseter major muscle, and possibly by the masseter zygomatica as well. The superior border of the root, differing in thickness, is amenable to similar influences by different portions of the masseter.

The anteorbital foramina are about three times as high as wide; but this means little, as it is impossible to measure them with accuracy. They do not vary appreciably except in the antero-posterior extent of the adjoining fossae upon the rostrum. Such differences are dependent upon the development of the anteorbital slip of the deep part of the masseter major. Width of rostrum is usually measured across the prominences anterior to the anteorbital fossae, but these are often fragile and easily injured, and it is an unreliable measurement on which to place dependence.

The shape of the incisive foramina is usually constant in the present series. These are not wide, and there is gradual and moderate, though definite, constriction posteriorly. In the case of normal adults and subadults, the length of the incisive foramina for males averages 5.5 mm., with extremes of 5.8 and 5, a difference of 0.8 mm., or 14.5 per cent of the average. Females average 5.35 mm., with extremes of 5.7 and 5, a difference of 0.7 mm., or 13 per cent. These foramina may be proportionately a trifle shorter and are situated relatively farther forward in juveniles, but after sexual maturity has been attained they vary hardly at all in comparison with the size of the entire skull. There are some examples in which the length of the foramina varies individually to some extent, but on the whole this measurement is quite uniform and dependable.

**PREMAXILLAE.**—Each of these bones articulates with the nasal, frontal, and maxilla. The ascending branch of the premaxilla projects posteriorly between the nasal and maxilla to join the frontal, and the resulting suture is somewhat variable, but not nearly to the extent that is sometimes to be found in other species. These branches are always pointed at the medial tips and differ negligibly in shape. Variation in the position of the posterior terminations with reference to the posterior terminations of the nasals is considerable, however. In 48 examples the ascending branches extend slightly farther backward while in 16 these may be considered as ending even with the nasals. It may or may not be fortuitous that among the latter large females are especially prevalent.

**NASALS.**—The nasals are quite variable, probably not from any inherent tendency to vary, but rather because of differences in the stresses brought to bear by surrounding bones. They articulate laterally with the premaxillae and posteriorly with the frontals. The measurement of length of nasals must be taken with extreme care in order to prove reliable. Not only is it sometimes difficult to decide upon the exact posterior terminations, but the anterior tips are exceedingly subject to injury. There is considerable individual variation in length of nasals and also much difference with age, these bones often being relatively shorter (and wider) in juveniles. In males the normal average of this measurement is 7.7 mm., with extremes of 8.8 and 6.9, a difference of 1.9 mm., or 24.7 per cent of the average. For females the average is 7.5 mm., with extremes of 8.3 and 6.9, a difference of 1.4 mm., or 18.6 per cent.

The posterior terminations of the nasals usually present a gently rounded aspect, although in a few they are more squarish, slightly emarginate, or so narrow

as to be almost pointed. It is in width, however, that this portion of the nasals chiefly varies, this difference in extremes amounting to fully 100 per cent. There is normally a slight lateral constriction at about the center of these bones, this being more pronounced in a few and absent in several others. The degree to which the anterior terminations of the nasals are arched, from an anterior aspect, is best shown by an examination of the anterior nares. Their outline varies greatly. In some extremes the height, measured from the nasal-premaxillary suture, is fully half the width of the opening, while at the other extreme are animals with nasals excessively flattened in which the height may be considered as being negligible. This latter type occurs in skulls having wide rostra, and a highly arched condition of the nares is undoubtedly caused by a medial crowding of the premaxillae in early life, due to some obscure stimulus. The degree to which the nasals project anteriorly also varies considerably, but in no case do those of adults hide the incisive processes of the premaxillae when the skull is viewed from above. In juveniles, however, in which the incisors are smaller and project to a lesser degree, the latter are barely visible from above.

# MANDIBLE

It was found that there is considerable variation in mandibular characters. The length over all from the condyle to tip of the incisor varies, of course, precisely as does the glenoid cavity to incisor measurement of the skull. The posterior portion of the mandible is essentially a system of levers and scaffolding for muscular insertions to even a greater extent than is the skull; hence its characters are exceedingly sensitive to differences in the variations of the muscles. These mandibular variations, however, are delicate rather than gross, and are of a sort difficult to express by measurements. The coronoid process varies in length, in width (antero-posteriorly), and in the degree to which it slopes rearward, and the same statements apply to the condyle, although variation in the latter is not correlated with that of the coronoid. There is great difference, irrespective of age, in the prominence of the slight process formed by the pulp capsule of the incisor (the incisive process of the condyle). In some examples this is obscure, while in others it is well developed. Its prominence is influenced partially, but not entirely, by the degree to which the condyle is bent inward, that is, out of plane with the remainder of the ramus. This, as well as the angle at which the whole supero-posterior portion of the ramus projects outward from the supero-inferior plane of the molars, varies in degree with the zygomatic width of the skull. The widest skulls usually, if not always, are accompanied by the most angular mandibles, often with prominent eminences at the roots of the incisors. The angular process, as well, usually follows such a trend. Its length, however, and its supero-inferior width vary independently of the other two processes, development being influenced by different muscles. The length of the angular process, measured from the prominence formed by the root of the posterior molar to its tip, is given in the tables; but neither this measurement nor that of any other portion of the mandible is deemed of great significance in systematic work with microtines when the accompanying skull is available.

The masseteric ridge does not vary individually to an appreciable extent and only very slightly with age. The coronoid process and the condyle are proportionately large in juveniles, but the angular process is relatively small. It has not been possible to correlate any of these variations save to the extent mentioned. Variation in the length of the teeth from one cause or another would have a corresponding effect upon the muscles of mastication and their insertions, but it is not known with any degree of certainty just what these are.

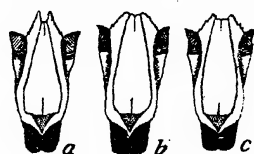


FIG. 21.—Individual variation of the nasals and ascending branches of the premaxillae in adult skulls. a, No. 3830; b, No. 3836; c, No. 3871

## THE TEETH

The changes that occur in the teeth of microtines soon after birth are exceedingly interesting and significant. As there are no newly born young in the present series, however, and data on dentition of this group are being presented in another paper under preparation by the author, this subject will not be treated in the present contribution.

Certain teeth, notably the last upper and first lower molars, are exceedingly variable, as is the rule in this subfamily. The tooth pattern of microtines is, of course, of very great value in diagnosing groups and many of the species. In spite of the efforts of many investigators, however, it has proved impossible to correlate minor variations either with age or any other character, and it is strongly felt that an undue importance has often, if not usually, been attached to such differences in enamel pattern in the study of closely related forms. There has been the same failure in the present study, and in spite of the author's best efforts along this line it has not been possible to assign to these variations within the series any significance whatsoever, save that they undoubtedly indicate some active, composite, evolutionary trends on the part of the teeth concerned.

There is considerable individual variation in the length of the maxillary tooth row, with age difference to correspond. The average length for males is 6.68

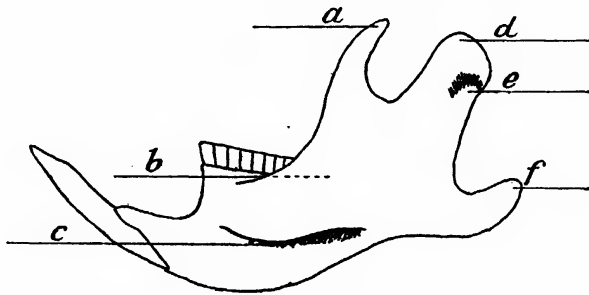


FIG. 22.—Diagrammatic lateral aspect of left ramus of the mandible of adult skull. *a*, Coronoid process; *b*, temporal fossa; *c*, masseteric ridge; *d*, condyle; *e*, incisive process; *f*, angular process

mm., with extremes of 7.2 and 6.1, a difference of 1.1 mm., or 16.4 per cent of the average. The average for females is 6.7 mm., with extremes of 7.4 and 6, a difference of 1.4 mm., or 20.9 per cent. The sexual difference in size in favor of the females, although possibly of some significance, is not sufficiently pronounced to be of much value. It is worthy of note, however, that the greater variation in this character occurs among the females instead of among males, as is usual in the case of most measurements. The size of the alveoli increases with general growth of the skull; hence the individual teeth are broader in both the antero-posterior and lateral dimensions in adults. The alveolar width, greatest distance between the lateral borders of the tooth rows, shows less individual variation, but almost as much variation with age as the length of tooth row. To a slight extent only is it correlated with the latter. In males it averages 5.61 mm., with extremes of 6.1 and 5.3, a difference of 8 mm., or 14.3 per cent. In females it averages 5.52 mm., with extremes of 6 and 5.4, a difference of 0.6 mm., or 10.8 per cent. In this character the greater size, as well as variation, occurs among males.

Variation in molar pattern is not by any means always bilaterally symmetrical, although corresponding teeth upon the two sides usually exhibit similar trends. There is no appreciable variation in the amount of dentine occurring within the

reentrant angles. In the smaller teeth of juveniles the angles may appear to be more acute and sharply defined than in adults, but this seems to be merely because of the difference in the size of the teeth and is not relative. There are, however, fluctuations in the exact form of the reentrant and salient angles, but these are haphazard and purely individual. If they have significance it is extremely obscure.

Any conclusions based upon the length of the teeth must be accepted with the greatest caution, for teeth are prone to become loose during cleaning of the skull and are then easily pushed in or pulled out beyond their normal positions. After drying, it is extremely difficult to detect such misplacement unless it be uneven. The length of the anterior molars in relation to the posterior ones, in both upper and lower series, is, of course, interdependent upon the length of the incisors and the configuration of both the upper and lower diastemata.

It is entirely permissible to presume that there is some slight fluctuation in the rate at which the teeth of different individuals grow from the persistent pulp, and there must also be variation in the rate at which the teeth are worn away, according to the habits of the individual and the character of the food consumed. Longer incisors, brought about by one of these causes, would therefore effect longer anterior cheek teeth, for these latter can not wear down more rapidly than the wear of the incisors permits.

INCISORS.—In the series at hand there is no relative variation in the incisors save in wear and size with age. As the skull increases in size so do the alveoli,

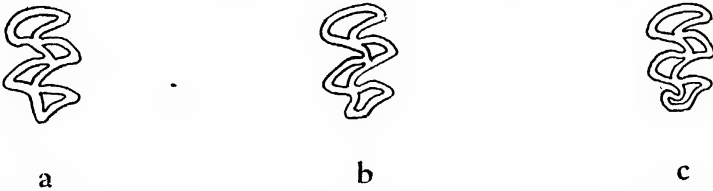


FIG. 23.—Individual variation in the pattern of the second upper molar M2:  
a, No. 3825; b, No. 3815; c, No. 3804

and consequently the size of the persistent pulp cavities. Hence the incisors of adults are of a larger diameter and greater arc of curvature with the projection of the free portion correspondingly greater. With increased use of the incisors with age there is greater wear upon their posterior faces. The cutting points of the lower incisors in adults, however, are practically as sharp (acute) as in immatures; hence the pits upon the upper incisors are but a shade wider in the older animals, often leaving a thin splinter of enamel projecting upon the lateral edge of the upper incisors of the larger skulls.

#### Maxillary Molars

FIRST MOLAR.<sup>5</sup>—This tooth is composed of the usual anterior, crescentic loop, two inner, and two outer triangles, all closed. There is no appreciable variation in its pattern.

SECOND MOLAR.—This consists of an anterior crescentic loop, an inner and two outer triangles, all closed. Only the posterior space is variable. At one extreme is a tendency toward acute sharpness of the posterior enamel faces (fig. 23, a), but this condition is rare, occurring to a marked extent in but two or three examples. At the other extreme is a condition in which the posterior triangle has developed a slight though definite postero-internal loop (fig. 23, c) or spur (metaconule), the enamel space remaining open. This character is well developed in but two individuals, both females, but there is a trace of it present in 18

<sup>5</sup> The usual terminology of first, second, and third molars is employed.

others—7 males and 11 females. Needless to say, the allocation of a number of examples in regard to the presence or absence of such a trace is purely arbitrary. As this tendency is present to a greater or lesser extent in at least 30 per cent of the skulls, it is of considerable importance. The pattern which may be considered as normal is illustrated in figure 23, *b*.

**THIRD MOLAR.**—The normal pattern of this tooth consists of an anterior crescentic loop, two external, and one internal closed triangles, and posteriorly, two open, internal loops, highly variable in this species as in most other microtines. In 33 individuals the penultimate loop is rather constricted, and the posterior one is subtended by a distinct, intero-posterior notch (fig. 24, *e*). In two or three cases this indentation is sufficiently pronounced so that, in reality, one might consider that there are five internal salient angles instead of the usual four. As hardly any two individuals can be found in which this portion of the third molar is precisely duplicated, the allocation of many specimens is arbitrary; but this general type may be considered normal, as it is the one more often found than any other. It insensibly grades into another subtype of pattern, represented by 26 individuals, in which the posterior enamel space has no marked indentation (fig. 24, *d*). In a third type, represented by 5 specimens, the internal salient angle of the posterior enamel space is definitely hooked forward (fig. 24, *c*). In two skulls the posterior enamel space is exceedingly small, with-

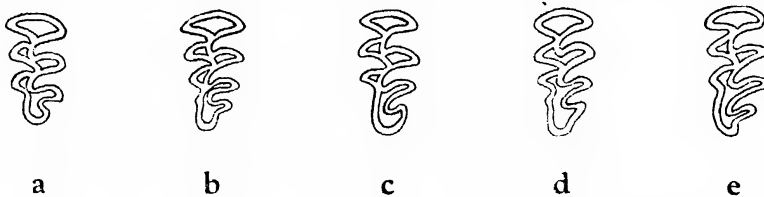


FIG. 24.—Individual variation in the pattern of the third upper molar (M3: *a*, No. 3817; *b*, No. 3873; *c*, No. 3864; *d*, No. 3837; *e*, No. 3835

out indentations (fig. 24, *a*), and in one (fig. 24, *b*) the penultimate loop upon the right side, but not upon the left, is entirely closed, a condition that may be considered as aberrant. These five types of enamel pattern may be still further subdivided at the pleasure of the investigator, but nothing is to be gained thereby. There is also a tendency exhibited by a few individuals for other triangles to remain open, as illustrated by figure 24, *d*, and the posterior internal triangle is found in this condition not infrequently.

It is entirely impossible to correlate these variational trends with age or any other character, and they can be considered only as evidence that the third upper molar is at present much more unstable in an evolutionary sense than either of the two other maxillary cheek teeth. This is the last of the upper teeth to emerge, and hence it is relatively less worn and developed in small juveniles than in older specimens.

#### Mandibular Molars

**FIRST MOLAR.**—As is the case with the majority of forms in this subfamily, the first lower molar is the most variable as well as the most complex of all the cheek teeth. It is in the anterior, tripartite enamel space that this diversity is encountered, and the variation is well nigh infinite. Truly no two are alike, nor is there bilateral symmetry between the two rami in this respect, for as often as not each ramus must be assigned to a different group. If it be attempted to establish too many groups for the different types of variants, however, a condition bordering on chaos results, with weakly-defined criteria for designation, and it is better to limit the number of main groups to three.

In the first group the outer lobe of the trisection is situated practically entirely posterior to the inner. All loops remain open, and each loop may vary widely in size and proportions. In this group, represented by figure 25, *a*, are 75 of the 132 rami.

In the second group, of which figure 25, *b* is typical, the outer lobe of the trisection is *not* practically entirely posterior to the inner, but in only a few cases is it situated just as far forward as the inner. All loops remain open in this group also, in which belong 48 of the rami.

Nine rami are assigned to the third group, illustrated by figure 25, *c* and *g*. In it the outer lobe of the trisection is situated posterior to the inner one and is entirely closed, forming a supplementary triangle upon that side of the tooth. In the remaining details of figure 25 are shown the more noteworthy variations of these three patterns.

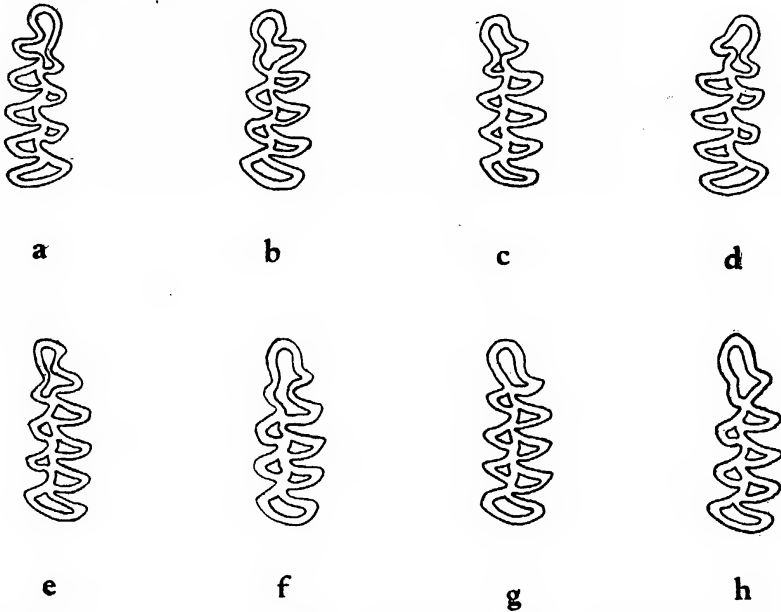


FIG. 25.—Individual variation in the pattern of the first lower molar.  $\overline{MI}$ : *a*, No. 3888; *d*, No. 3873;  $\overline{MI}$ : *b*, No. 3850; *c*, No. 3820; *e*, No. 3842; *f*, No. 3853; *g*, No. 3825; *h*, No. 3863

The writer is convinced that this great variation in tooth pattern within the series is without significance in the present connection, save, as in the case of the third upper molar, that it indicates evolutionary instability at this point. As previously mentioned, additional subgroups might be designated in order to classify the variants with still finer exactness, but as the differences are purely individual and without connection with other characters, in so far as it is possible to see, they need not be dwelt upon in greater detail in the present paper.

The first lower molar, then, consists of a highly variable, tripartite division anteriorly, followed by two closed triangles upon the outer or buccal side and three upon the inner or lingual side, with a posterior crescentic loop. All these triangles are normally closed, exceptions occurring in two cases in which the anterior inner triangle remains open (fig. 25, *f*).

SECOND MOLAR.—This consists of two small outer triangles, two larger inner ones, and a posterior crescentic loop. There is no appreciable variation.

THIRD MOLAR.—In this tooth there are three simple, closed loops, the anterior one smallest and the other two progressively larger. The inner reentrant angles are deep, but the outer ones are barely indicated. There is no appreciable variation.



## SUMMARY

This paper embodies the results of an intensive study of a large series of a meadow mouse, *Microtus montanus yosemite* Grinnell, undertaken to secure data on the amount of variation to be found in a single species of small mammal represented by specimens taken at the same place, within a short period, and by the same collector, thus insuring uniformity as regards preparation of material. It is believed that the results will furnish a criterion useful to later students of related groups of small rodents.

The series under discussion, consisting of 66 skins with skulls, besides spirit specimens, was collected at virtually one time and at a single spot, in Mono County, California. The conditions existing there were very uniform, and the locality unusually free from disturbing influences.

Coloration, especially of the females, is somewhat variable. Much of the difference in the appearance of the pelage of males is due to fighting and to varying development of the hip glands. The females may be divided into a grayish group, in which are most of the large adults, many of which are grayer than any male; and a brownish one, consisting largely of subadults. Juveniles have a pelage that is more compact and slightly darker.

The molt is insidious. Four females are excessively worn, but there are no males in this condition.

The plantar tubercles are variable in size and number, but five is the usual complement. Hip glands vary in development, those of old females being of about the same size as in subadult males. They are absent in juveniles and are probably dependent upon sexual development. There are eight mammae present.

The measurement of total length is subject to considerable error, and the variation in sexually mature animals is great. The tail not only varies much in length, but it is especially subject to injury during the life of the animal. The hind foot attains full growth at an early age, and its length, although varying to some extent, is a reliable character.

Weight varies a great deal, especially in the case of females, as the latter often contain embryos, but it is very valuable as an index to the general development of the animal. Young females are more often found to be pregnant than old ones, but with the latter the litters average larger.

Most of the individual variation of the skull is due to differences in the development of the muscles of mastication, and no thorough understanding of the osteology can be gained without some knowledge of the myology. Bones to which the main muscles are attached vary in response to stimulation by the latter. Other bones vary because of crowding.

The skull consists of the face and cranium proper, the latter being divisible into three rings, the anterior one of which is "dead center" of the skull. Skulls of juveniles are much weaker and more rounded than those of adults, and this, with lack of ridging in the former, accentuates the appearance of age difference to a greater extent than is actually found to be the case. The capacity of the braincase is, to all intents, proportionately the same at all ages.

The measurement of condylo-basilar length is the most satisfactory criterion for the arrangement of a series of skulls with respect to general development. Other composite measurements are of value as indices to the variation existing in divers portions of the skull and to indicate correlated trends. They vary in different degrees, both individually and with age. Variation in the precise configuration of the bones of the skull is, of course, infinite, such being the result of an enormous number of the most complicated developmental factors, only a very few of which can be partially defined.

Variation of the mandible is considerable, but the characters are usually correlated with other portions of the skull, and it is considered that most mandibular characters are relatively unimportant in systematic work when the skull proper is available.

The patterns of the first lower and third upper molars are excessively variable, but this is individual, and practically no part of it is due to age. It is felt that an undue phylogenetic importance has usually been ascribed to these differences within a group of species, but that in such aggregations of individuals as the present they point to nothing more than unstable evolutionary trends.

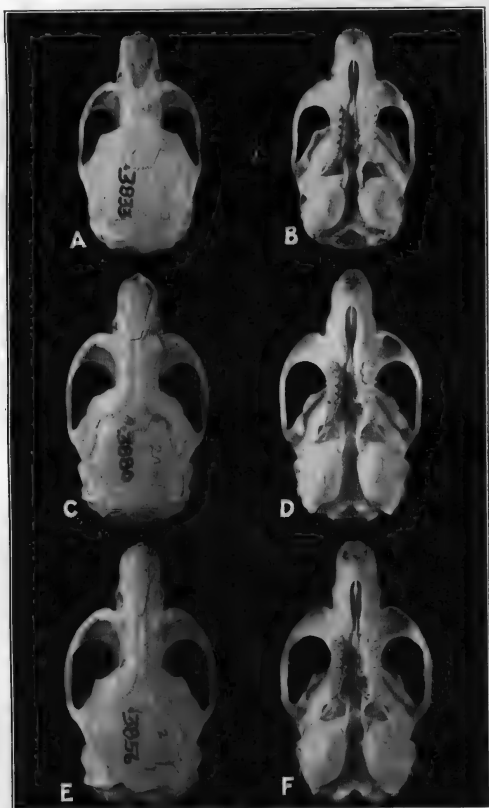
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PLATE 1

Dorsal and ventral aspects, twice natural size, of adult No. 3856, immature No. 3880, and juvenile No. 3833, of *Microtus montanus yosemite* Grinnell, showing variation with age.

(1016)



# A GENETIC AND CYTOLOGICAL STUDY OF CERTAIN HYBRIDS OF WHEAT SPECIES<sup>1</sup>

By KARL SAX, *Biologist, Maine Agricultural Experiment Station*, and E. F. GAINES, *Cerealist, Washington Agricultural Experiment Station*

The cultivated species of wheat can be divided into three definite groups based on genetic, taxonomic, and cytological classification. Each of these groups has certain specific characteristics not found in the other groups. The Einkorn group is of no economic importance. The emmer group is of considerable economic importance in certain wheat-growing districts, while the vulgare group includes most of the varieties in common use. The emmer group in general possesses many desirable characteristics such as disease resistance, drought resistance, high protein content, and high yield under certain conditions. The vulgare group, on the other hand, is in general susceptible to disease and in most cases is not adapted to a semiarid climatic condition, but the only varieties of wheat which produce a high quality of grain are in this group. It is only natural that for many years plant breeders have attempted to combine the desirable characters of the emmer wheats with the high quality of the vulgare wheats. It would seem to be a simple problem to combine, for instance, the disease and drought resistance of the durum wheat with the high yield and bread-making qualities of certain vulgare wheats, but apparently no economic varieties have been obtained by combining the desirable characteristics of these two classes of wheat species. In the progeny of such crosses certain parental characters were found to be associated together, and it was difficult, or perhaps impossible, to obtain the desirable combinations in a single individual.

Crosses of wheat varieties which belong to different groups result in partially sterile  $F_1$  plants and different degrees of sterility in  $F_2$ . Crosses between different species of the two groups result in different degrees of sterility. Some combinations show a very high degree of sterility while others are nearly as fertile as the parents.

A study of the chromosome behavior in such partially sterile hybrids has shown the reason for the sterility and why certain characters are more or less closely associated in the  $F_2$  hybrids. The wheat species in the emmer group have 14 haploid chromosomes while those in the vulgare group have 21 haploid chromosomes. On crossing species of these two groups with different chromosome numbers an  $F_1$  plant is obtained with 35 chromosomes in the somatic cells. In the reduction division of the  $F_1$  there are 14 pairs of chromosomes and 7 single chromosomes. The 14 pairs of chromosomes undergo reduction in the usual manner, but the 7 single chromosomes are apparently distributed at random to one pole or the other, so that the gametes will contain from 14 to 21 chromosomes (10).<sup>2</sup> In most cases, at least, the perfection of the gametes increases as the chromosome number approaches 14 or 21, and the gametes with certain intermediate numbers are eliminated, due to sterility. Genetic investigations conducted in

<sup>1</sup> Received for publication Mar. 31, 1924—issued Nov., 1924.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," pp. 1031-1032.

connection with cytological work indicates that the 7 additional chromosomes possessed by the vulgare group determine in some way the characteristic properties of that group. Thus it would be expected that segregates of such partially sterile hybrids would resemble the vulgare wheat if the segregates possess 21 chromosomes, while the 14 chromosome segregates would resemble the emmer group. The association of certain characters with chromosome number in  $F_2$  would explain why certain characters are usually associated and why desirable characters of the two groups of species can not readily be combined.

Since there is considerable difference in the degree of sterility of certain species crosses it might be expected that the segregates from comparatively sterile crosses would tend to resemble more closely the parental form, while with increased fertility certain segregates might be obtained which would combine certain of the desirable properties of the two parental species. It may also be possible to determine the location of certain genetic factors in these hybrids. If, for instance, certain characters are determined by factors in the 14 primary chromosomes which pair in the species crosses, then these characters would be expected to segregate more or less normally. On the other hand, characters borne by the 7 additional chromosomes of the vulgare wheat would be expected to behave in a nonMendelian manner, due to the random distribution of a single set of chromosomes and more or less sterility of gametes and segregates with an intermediate chromosome number. Thus it should be possible to determine whether certain genetic factors are in the 14 primary chromosomes or are in the additional chromosomes of the vulgare species. A study has been made of the sterility, genetics, and cytology of a number of such species crosses with these objects in view.

### MATERIAL AND METHODS

The parents used in this investigation include three species in the emmer group and two species in the vulgare group, as follows:

	Variety	Agronomic variety
EMMER GROUP		
<i>Triticum polonicum</i> L.	<i>villosum</i> Körn	Polish.
<i>T. turgidum</i> L.	<i>pseudo-cervinum</i> Körn.	Alaska.
<i>T. Durum</i> Desf.	<i>hordeiforme</i> Körn.	Kubanka.
VULGARE GROUP		
<i>T. vulgare</i> Vill.	<i>lutescens</i> Körn.	Marquis.
<i>T. vulgare</i> Vill.	<i>albidum</i> Körn.	Amby.
<i>T. compactum</i> Host.	<i>humboldtii</i> Körn.	Washington Hybrid 143.

In describing and referring to the varieties the agronomic name will be used. Spikes of the different varieties are shown in Plate 1. Following is a brief description of the varieties used. Alaska is a spring wheat, with branched spike, black awns 3 to 12 cm. long, kernels white and usually starchy. Polish is a spring wheat with black awns 4 to 10 cm. long, glumes and kernels very long, awns usually deciduous, kernels white, and very hard. Kubanka is a spring wheat with glumes and awns yellowish, awns from 6 to 15 cm. long, kernels white and very hard. These three varieties all belong to the emmer group and all have more or less pithy straw and sharply keeled glumes.

The varieties belonging to the vulgare group in all cases have hollow straw and the keel on the back of the glume is relatively small. Marquis is an early spring wheat and has yellowish glumes, hard, red grain, is considered awnless, but has a few apical awns 1 to 10 mm. long. Amby is an Australian wheat which is completely awnless and has white glumes and white kernels. Washington Hybrid 143 is a club wheat with very soft grain and apical awns 2 to 10 mm. long.

Crosses were made between Marquis  $\times$  Kubanka, Hybrid 143  $\times$  Alaska, Marquis  $\times$  Alaska, Hybrid 143  $\times$  Polish and Amby  $\times$  Kubanka. The  $F_2$  generation of each cross was grown at the Washington Agricultural Experiment Station, Pullman, Wash, in 1921. Conditions in eastern Washington are ideal for growing wheat for genetic studies, because the semiarid climate permits maturity without any discoloration of glumes or awns and the color characters can be studied readily. The percentage of segregates which will germinate and mature is also much higher than is the case in the Middle Western or Eastern States. Data on height, time of maturity, and per cent of sterility of the  $F_1$  plants were obtained at the Washington Experiment Station. One head from each  $F_2$  and  $F_3$  plant was sent to the Maine Agricultural Station for a more detailed classification. A code was made for most characters, and all data were punched on cards so that the material could be analyzed with the aid of a sorting machine.<sup>3</sup>

In certain crosses each character was correlated with every other character to determine the degree of association between the different characters. The correlation ratio, the correlation coefficient, and the coefficient of contingency were used, depending on the nature of the data. In obtaining correlations between  $F_2$  and  $F_3$  the mean values of the  $F_3$  families were used in most cases.

Certain of the intermediate types in  $F_3$  were selected and grown for a cytological study in  $F_4$ .

### STERILITY IN SPECIES CROSSES

The degree of sterility in each of the five crosses is shown for the different generations. In the  $F_1$  of each cross the actual number of grains per spikelet was used, but in  $F_2$  and  $F_3$  only the grains in the two outer florets were considered, so that two grains per spikelet would be complete fertility. In the parent varieties the grains per spikelet ranged from about 1.7 to 2.

<sup>3</sup> The following code was used in describing  $F_2$  and  $F_3$  segregates:

- |  |   |
|--|---|
| <p>A. Type of culm—</p> <ol style="list-style-type: none"> <li>1. Hollow.</li> <li>2. Intermediate.</li> <li>3. Solid.</li> </ol> <p>B. Diameter of culm in millimeters.</p> <p>C. Type of spike—</p> <ol style="list-style-type: none"> <li>1. Open.</li> <li>2. Intermediate.</li> <li>3. Compressed.</li> </ol> <p>D. Type of glume—</p> <ol style="list-style-type: none"> <li>1. Slightly keeled.</li> <li>2. Medium keeled.</li> <li>3. Strongly keeled.</li> </ol> <p>E. Shape of glume—</p> <ol style="list-style-type: none"> <li>1. Square.</li> <li>2. Medium.</li> <li>3. Pointed.</li> </ol> <p>F. Color of glume—</p> <ol style="list-style-type: none"> <li>1. Yellow.</li> <li>2. Brown.</li> <li>3. Black.</li> </ol> <p>G. Outer glume pubescence—</p> <ol style="list-style-type: none"> <li>1. Absent.</li> <li>2. Slight.</li> <li>3. Heavy.</li> </ol> | <p>H. Awned. Ranging from absent to those more than 10 cm. long. In <math>F_3</math> the actual length of the longest awn was recorded.</p> <p>I. Awn color—</p> <ol style="list-style-type: none"> <li>1. Yellow.</li> <li>2. Brown.</li> <li>3. Black.</li> </ol> <p>J. Length of rachis in centimeters.</p> <p>K. Texture of rachis—</p> <ol style="list-style-type: none"> <li>0. Strong.</li> <li>1. Intermediate.</li> <li>2. Brittle.</li> </ol> <p>L. Number of spikelets.</p> <p>M. Number of grains.</p> <p>N. Number of grains per spikelet.</p> <p>O. Grain length in millimeters.</p> <p>P. Grain shape.</p> <p>Q. Grain color, ultimately classified as either white or red.</p> <p>R. Grain texture, starchy, intermediate, and very hard.</p> <p>T. Type of head. Segregates were classed according to their resemblance to the different species of wheat, <i>vulgare</i>, <i>durum</i>, <i>spelta</i>, etc.</p> |
|--|---|

It is evident that there is considerable range in sterility of  $F_1$  hybrids. In the cross Marquis  $\times$  Alaska the  $F_1$  plant was practically as fertile as either parent. The only indication of sterility was the wrinkled grain and the great range in size of the kernels. In  $F_2$  the range in average sterility for the different crosses is less than the  $F_1$ , due partly to the greater number of individuals present. There seems to be little relation between sterility in  $F_1$  and sterility in  $F_2$ . This result is not entirely unexpected since in the more sterile  $F_1$  individuals only the more favorable combinations would survive, while in such crosses as Marquis  $\times$  Alaska many of the gametic recombinations would survive and function more or less favorably. In the  $F_2$ , sterility is due not only to gametic incompatibility but also to differences in somatic development. Certain plants are so poorly developed that they set no grain, while others are extremely vigorous in vegetative development but are sterile, due to gametic imperfections. The difference in sterility in  $F_3$  is significant. In the case of Marquis  $\times$  Alaska the sterility is greater than in the cross Amby  $\times$  Kubanka. This is partly due to climatic conditions, since the  $F_3$  of Marquis  $\times$  Alaska was grown in Washington State where a greater number of the weaker types undoubtedly survived, while the other cross was grown in Maine and only the strongest and most vigorous segregates could survive the extreme climatic conditions.

TABLE I.—*Sterility of various species crosses as indicated by average number of grains per spikelet (GS) in  $F_1$ ,  $F_2$ , and  $F_3$ . The number (n) of individuals involved is indicated in each case*

Crosses	$F_1$		$F_2$		$F_3$	
	n	GS	n	GS	n	GS
Marquis $\times$ Kubanka	13	$1.05 \pm 0.08$	135	$0.80 \pm 0.03$		
Hybrid 143 $\times$ Alaska	34	$1.09 \pm .05$	293	$.63 \pm .02$		
Marquis $\times$ Alaska	8	$2.00 \pm .16$	152	$.98 \pm .03$	1536	$1.09 \pm 0.01$
Hybrid 143 $\times$ Polish	19	$.66 \pm .06$	82	$.70 \pm .04$		
Amby $\times$ Kubanka	8	$.58 \pm .03$	52	$1.07 \pm .06$	38	$1.59 \pm .04$

It has been found that gametic and somatic sterility is closely associated with chromosome behavior. Those individuals with their chromosome number approaching either 14 or 21 survived more frequently than the individuals with an intermediate chromosome number. Thus it would be expected that where there was a high degree of sterility in  $F_1$  the segregates with an intermediate chromosome number would be more rapidly eliminated, while in the relatively fertile combinations segregates with an intermediate number might survive in many cases and a relatively high degree of sterility would persist for a number of generations, although complete sterility would be comparatively rare. This hypothesis can probably be checked by an analysis of genetic behavior. For instance, in the more sterile combinations one might expect to find a higher correlation between the various characters due to the elimination of intermediate types. In such a case the surviving segregates would resemble the emmer parent or the vulgare parent and the characteristic features of the two groups would be more or less closely associated. In less sterile individuals the intermediate type would be expected to survive to a considerable extent, so that the vulgare and emmer characteristics might be combined in many cases and there would then be little correlation between the characteristic features of the two groups. One might also expect the recovery of occasional segregates combining the desirable characters of the two species in the more fertile combinations, while in more sterile hybrids the only homozygous fertile segregates would resemble one parent or the other. The available data do not, however, indicate



that segregates with a chromosome number intermediate between 14 and 21 can be obtained in a completely fertile and homozygous condition.

### GENETIC INHERITANCE IN SPECIES CROSSES

Most of the characters studied in these various crosses do not appear to segregate in a simple Mendelian manner, and the percentage of homozygous individuals resembling one parent or the other are in most cases comparatively small. In the case of the cross Marquis  $\times$  Alaska, the  $F_2$  classification has been checked by growing the  $F_3$  and the same general conclusions have been verified. There are, however, two or three characters which seem to segregate in the usual Mendelian manner, indicating perhaps that these characters are dependent on factors in the 14 primary chromosomes which behave in a normal manner in the reduction division. Some of the more typical characters are shown in Table II, to illustrate the inheritance of those characters which seem to segregate in a Mendelian manner and those which show no clear segregation into definite Mendelian ratios.

TABLE II.—*Segregation of certain characters in  $F_2$  of various crosses of wheat species*

Cross	Length of awns in centimeters					
	Tips			4-10	10+	n
	1	2-3	4-10			
1. Marquis $\times$ Kubanka.....	48	53	9	17	8	135
2. Hybrid 143 $\times$ Alaska.....	188	36	8	57	3	292
3. Marquis $\times$ Alaska.....	65	42	14	14	17	152
4. Hybrid 143 $\times$ Polish.....	48	11	5	13	5	82
5. Amby $\times$ Kubanka.....	36	10	-----	2	4	52

### TYPE OF KEEL

Cross	Vulgare	Inter- mediate	Durum	n
1.....	36	55	44	135
2.....	86	87	120	293
3.....	38	85	29	152
4.....	7	56	19	82
5.....	15	26	11	52

### AWN COLOR

Cross	Yellow	Brown	Black	n
2.....	105	56	84	245
3.....	77	13	62	152

### GRAIN TEXTURE

Cross	Very hard	Hard	Inter- mediate	Starchy	Very starchy	n
1.....	50	63	3	1	-----	117
2.....	24	51	-----	77	47	199
3.....	24	76	36	9	2	147
4.....	21	41	2	-----	4	68
5.....	1	26	6	9	7	49

In the description of the various  $F_2$  segregates the length of the awn was arbitrarily grouped into five classes as indicated in Table II. Individuals with awns from 1 to 3 cm. long when awned only at the tip would ordinarily be classed as awnless segregates. Those with awns from 4 to 10 cm. in length when found at the tips only might be considered in some cases as intermediate, although the awnless condition is more or less dominant in wheat crosses. Segregates with awns from 4 to 10 cm. in length resembled the awned vulgare wheat, while individuals with awns greater than 10 cm. in length could be classed as the emmer type of awn. In the first four crosses there is a rough approximation to a 3:1 ratio of awnless to awned. In the fifth cross, Amby  $\times$  Kubanka, the percentage of awned individuals is relatively low. Amby is a completely awnless wheat and the Howards (8) have found that a true awnless wheat crossed with an individual with long awns results in a 15:1 ratio. In this cross of Amby  $\times$  Kubanka the ratio more closely approximates the 15:1 ratio than it does the 3:1 ratio.

In general, segregates of crosses between emmer and vulgare wheats can be classified as the emmer type or vulgare type according to the shape of the keel. In the  $F_2$  segregation of keel type is apparently dependent on a single Mendelian factor in some cases, but the classification is more or less arbitrary and the  $F_3$  behavior does not indicate a single factor difference for this pair of characters.

In the crosses involving Alaska it was possible to classify the segregates according to color of awn. Even in the so-called awnless segregates the length of the awn was sufficient to indicate the color present. The classification of awn color is very difficult because climatic conditions in many cases apparently inhibit the development of the black color, and even in parental varieties the color may appear in some individuals and be absent in others. The classification of certain individuals as brown is not definite but simply indicates that they could hardly be classed as yellow or black. Although the  $F_2$  segregation does not indicate any simple Mendelian segregation, the  $F_3$  generation of Marquis  $\times$  Alaska does indicate a single factor difference.

Grain texture is one of the characters which is unusually difficult to classify. The texture of the grain was determined in most cases from a single head of each plant in  $F_2$  and  $F_3$ . Subsequent work during the past winter has shown that on a single plant certain heads may bear grain, all of which would rank as hard, while other heads of the same plant would have only soft grain. This character is apparently dependent on environmental conditions to such an extent that no satisfactory genetic analysis can be made of this character, at least in these species hybrids. In most of the crosses described the emmer parent had extremely hard grain, while the vulgare parent has relatively soft grain. The condition is apparently reversed in the case of Marquis  $\times$  Alaska, however, because the Marquis has comparatively hard grain, while Alaska has a starchy grain due apparently to the factor for yellowberry. When the factor for yellowberry in Alaska is eliminated Alaska has grain practically as hard as the other species in the emmer group. This is indicated by the fact that flinty grains are found in certain segregates in  $F_2$  and  $F_3$ .

The cross Marquis  $\times$  Alaska was carried to the  $F_3$ , and 1,536  $F_3$  individuals were analyzed. A study of the various  $F_3$  families indicates the true genetic nature of the  $F_2$  segregates and permits an accurate classification of the  $F_2$  generation. For most characters 110  $F_2$  segregates are represented in  $F_3$ , although for certain grain characters fewer individuals are available, due to partial or complete sterility in  $F_3$ . The  $F_2$  behavior, as indicated by  $F_3$  segregation, is shown in Table III.

TABLE III.—*Marquis* × *Alaska*— $F_2$  behavior as indicated by  $F_3$  segregation; showing the number of  $F_3$  families which breed true for the alternative characters, and the number which show segregation

Characters studied	Behavior of $F_2$ families			n
Awning.....	Awnless.	Segregating.	Awned.	
Actual number.....	28	51	31	110
Theoretical for 3:1 ratio.....	27.5	55	27.5	
Awn color.....	Yellow.	Segregating.	Black.	
Actual number.....	26	66	18	110
Theoretical for 3:1 ratio.....	27.5	55	27.5	
Grain texture.....	Hard.	Segregating.	Soft.	
Actual number.....	10	80	8	98
Spike shape.....	Normal.	Segregating.	Branched.	
Actual number.....	17	89	4	110
Type keel.....	Vulgar.	Segregating.	Durum.	
Actual number.....	13	96	1	110
Grain color.....	Red.	Segregating.	White.	
Actual number.....	32	56	6	94

If no segregation occurred in seven or eight or more  $F_3$  individuals of a certain family the  $F_2$  parent was considered homozygous for the character involved (assuming a single factor to be involved). In this way the different  $F_2$  individuals could be classed as homozygous for one character or the other or heterozygous as indicated by  $F_3$  segregation.

When all  $F_3$  plants of a given family had an awn length of less than 3 cm. the  $F_2$  was considered homozygous for the awnless condition. It was found that all of the  $F_2$  individuals in the first two classes bred true for awnless or segregated into awned or awnless in the  $F_3$ . Some of the individuals are classed as having awns from 4 to 10 cm. long on the tips in the  $F_2$  bred true for awns in  $F_3$ , while a few of them segregated into awned and awnless. All of the  $F_2$  individuals which had awns 4 cm. and longer distributed the entire length of the spike bred true for the awned condition in  $F_3$ . The segregation of the awned condition as shown in Table III clearly indicates a simple one-factor difference for so-called awnless and awned segregates in  $F_2$ . This behavior is in accord with results found in varietal crosses (4, 7, 8, 9).

Although it is practically impossible to accurately classify awn color in  $F_2$  due to the effect of environmental factors, it is comparatively easy to classify the  $F_2$  segregates based on  $F_3$  behavior. The effect of environmental factors is undoubtedly responsible for the excess of individuals classified as segregating. No doubt a certain number of these individuals are genetically black, but climatic conditions in some way inhibit the black color from appearing in certain individuals, so the  $F_3$  family was classed as segregating into black and yellow. There is, however, little question but that here again a simple factor for black versus yellow or brown is involved.

A great majority of  $F_3$  families showed segregation for grain texture. Only 10 individuals were homozygous for hard grain and 8 homozygous for soft grain. There is, apparently, no simple Mendelian factor difference involved in this case.

Individuals were also classified according to shape of spike, whether normal or branched. In the  $F_3$  there were several individuals which were quite similar to the *Alaska*, at least in the morphological appearance of the spike. In  $F_2$  five segregates out of 152 were classed as branched while 48 were classed as partially branched, that is, showed some tendency of branching of a few spikelets. It is evident, then, that the normal condition is partially dominant over the branched condition. All of the completely branched individuals in  $F_2$  which resembled the *Alaska* parent to some extent, bred true in  $F_3$ . There is in this case no indication of simple Mendelian segregation.

Only one  $F_3$  family bred true for the emmer type of keel, while 13 families were homozygous for the vulgare type of keel. Most of the individuals were apparently heterozygous and there is no indication of a simple ratio.

Grain color is a very difficult character to classify in  $F_2$ , due to different degrees of hardness of the grain. A very hard endosperm will give the grain an amber appearance, which may sometimes be mistaken for red. However, the  $F_3$  behavior indicates the true genetic composition of the  $F_2$  individuals and the classification of  $F_3$  families into homozygous and heterozygous types is less difficult. All individuals classed as light amber in the original code have been considered as white in the present classification. The segregation approximates very closely that expected for a two-factor difference, the number of homozygous whites being practically 1 in 16  $F_2$  segregates. If two factors for red grain are involved in this cross, however, there should be a much larger proportion of the  $F_3$  families breeding true for red. There should be forty-two  $F_3$  families breeding true for red and forty-eight families segregating either into 15 : 1 or 3 : 1. Also due to the paucity of the individuals in  $F_3$  families, the twenty-four families which should show a 15 : 1 segregation would in many cases be classed as homozygous for at least one factor for red, so that the proportion of red should be in excess of 42, which does not agree with the actual data.

The  $F_2$  segregates were also classified in respect to type of head, whether they resembled *vulgare*, *compactum*, *Spelta*, etc. The types which bred true in the third generation included 4 *vulgare*, 1 *Spelta*, 2 *durum*, 2 *dicoccum*, and 1 classed as abnormal. There were also two *compactum* segregates in  $F_2$  which bred true to the type in  $F_3$ , but it is probable that these plants were mixtures, as they were completely fertile in all cases in both  $F_2$  and  $F_3$  and were extremely uniform in all characters.

The date of ripening in  $F_3$  ranged from the 10th of August to the 9th of September. Only eighteen  $F_3$  families were apparently homozygous for date of ripening. Fourteen families which were homozygous ripened between the 10th and 13th of August while most of the heterozygous segregates had a mean date of ripening ranging from August 13 to September 9. There was some correlation in  $F_3$  between type of spike and date ripe. The *Spelta* segregates ripened earlier than the abnormal types which could not be classified.

A number of correlations between various factors were obtained in  $F_3$ , and between the  $F_2$  individuals and  $F_3$  families of the cross Marquis  $\times$  Alaska. Segregates in  $F_2$  which were comparatively sterile were also relatively sterile in  $F_3$  while the more fertile  $F_2$  individuals resulted in comparatively fertile progeny in  $F_3$ . The correlation between grains per spikelet in  $F_2$  and grains per spikelet in  $F_3$  was  $.54 \pm .05$ . There was also considerable correlation between the various characters of  $F_2$  and  $F_3$  segregates. For instance, culm type in  $F_2$  correlated with culm diameter in  $F_3$  gave a correlation ratio of  $.59 \pm .04$ . Type of keel in  $F_2$  correlated with keel type in  $F_3$ , using the coefficient of contingency, gave a value of  $.52 \pm .06$ . Height of plants in  $F_3$  correlated with date ripe in  $F_3$  gave a value of  $r = -.18 \pm .06$ , indicating that vegetative vigor had little relation to the date of maturity. There was some tendency for the more fertile  $F_3$  segregates to ripen earlier, as indicated by the value of  $r = -.25 \pm .06$ . In these various cases the mean value of the  $F_3$  families was taken. Using all of the 1536  $F_3$  segregates a correlation of  $.62 \pm .01$  was found between grains per spikelet and height, indicating that fertility increased as the vegetative vigor increased and that much of the sterility was associated with poor vegetative development.

It might be expected that  $F_3$  segregates resembling the parent would be somewhat more fertile than intermediate or abnormal types. In Table IV the various types of segregates are shown, the number of such segregates, and the average

number of grains per spikelet. Segregates resembling the Alaska parent are the most fertile, followed by those resembling the vulgare parent. The intermediate types which could be placed in no particular group were the most nearly sterile.

TABLE IV.—*Segregation and average fertility of various types of F<sub>3</sub> segregates in Marquis × Alaska*

Type head	Number	Grains per spikelet
1. Vulgare.....	426	1.43
3. Spelta.....	155	1.18
4. Durum.....	307	1.23
5. Turgidum.....	49	1.70
6. Emmer.....	154	1.33
9. Abnormal.....	429	.81

The various types of segregates obtained in the F<sub>2</sub> of Marquis × Alaska and in the cross Hybrid 143 × Polish are shown in Plate 1. In the first case segregates are obtained which resemble either parent and also individuals which resemble *T. spelta* and *T. durum*. Types resembling *T. compactum* were also obtained but these may be due to mixtures. In the second cross types similar to *T. durum* were obtained as well as a number of types unlike any cultivated species. The segregates with very long glumes but with short heads as shown in Plate 1, C, b were found to be quite sterile in most cases.

#### THE ASSOCIATION OF VARIOUS CHARACTERS IN SPECIES HYBRIDS

It has been pointed out that in the more sterile hybrids the intermediate types would be more rapidly eliminated and the surviving segregates in F<sub>2</sub> and F<sub>3</sub> might resemble the parental types more or less closely. In more fertile crosses, on the other hand, the intermediate types might survive for several generations and gradually become eliminated only after a number of years. Since certain characters are found only in the emmer wheats and other characters are found only in the vulgare wheats one might expect a higher degree of association of these characters in F<sub>2</sub> segregates of relatively sterile hybrids, while there would be comparatively little association of parental characters in the more fertile F<sub>2</sub> segregates due to the fact that the intermediate types are not so rapidly eliminated. Thus, for instance, in one cross the F<sub>2</sub> and F<sub>3</sub> segregates consist largely of individuals with approximately 14 or 21 chromosomes, and there would be little opportunity for combinations to occur possessing characteristics of both parental groups. If, on the other hand, the cross resulted in an F<sub>2</sub> progeny which was relatively fertile and included many types with an intermediate chromosome number, then there would be more or less combination of parental characters and less association between the characters peculiar to each group.

TABLE V.—*Correlation between various characters of 52 F<sub>2</sub> segregates of Amby × Kubanka and of 152 F<sub>2</sub> segregates of Marquis × Alaska*

Characters correlated	Amby × Kubanka	Marquis × Alaska
Type of culm and—	<i>Correlations</i>	<i>Correlations</i>
Type of keel.....	0.86±0.03	0.24±0.05
Shape of spikelet.....	.90±.03	.23±.05
Awn length.....	.80±.04	.27±.05
Grain texture.....	.68±.07	.48±.05
Type of keel and—		
Shape of spikelet.....	.84±.03	.39±.05
Awn length.....	.74±.04	.30±.05
Compactness of head.....	.24±.09	.26±.05
Grain texture.....	.80±.03	.17±.05
Grain shape.....	.90±.02	.12±.05
Shape of spikelet and—		
Grain shape.....	.89±.02	.07±.05
Grain texture.....	.69±.05	.28±.05
Length of awns and—		
Grain shape.....	.36±.08	.23±.05
Grain texture.....	.49±.07	— .13±.05
Grains per spikelet and—		
Type of culm.....	.40±.08	.11±.05
Type of keel.....	.53±.07	.32±.05
Homozygous factors.....	.42±.08	.03±.05
Do.....	— .02±.09	.03±.05
Heterozygous factors.....	— .19±.09	— .08±.05

Correlations between various characters of a comparatively sterile cross, Amby × Kubanka, and a comparatively fertile cross, Marquis × Alaska, are shown in Table V. The various characters, type of culm, type of keel, shape of spikelet, and in most cases awn length and grain texture, are all characters which differ greatly in the emmer and vulgare groups and these characters in each group are more or less characteristic and are not found in varieties within the other group. In the cross Amby × Kubanka the correlation between the various characters is rather high in all cases, indicating considerable association of the parental characters. In the more fertile cross, Marquis × Alaska, the correlations are in most cases relatively low or are of little or no significance. In the correlation between type of culm and grain texture the correlation is relatively high. Although Marquis has a harder grain than Alaska when grown in eastern Washington, the durum and emmer segregates usually had flinty kernels harder than those of Marquis. This is due to the fact that Alaska has hard grain if the factors for "yellowberry" are absent. These correlations show clearly that in the more sterile hybrids the parental characters are more or less closely associated, while there is less association as fertility increases. It is possible, of course, that ultimately the more fertile hybrid would result in segregates resembling one parent or the other, and that the elimination of intermediate types occurs more slowly in the more fertile combinations. This is indicated by the degrees of sterility found in various types of segregates, as shown in Table IV. The abnormal types which do not resemble any of the economic species are the most sterile while segregates resembling the parent species are the most fertile.

One might expect the segregates resembling the parent to be more fertile than the intermediate types. This is found to be the case in the cross Amby × Kubanka, as indicated by the correlation between grains per spikelet and type of culm. The culm type and keel type resembling these parental forms are associated with comparatively fertile individuals, while the intermediate forms are associated with relatively sterile individuals in F<sub>2</sub>. In the cross Marquis × Alaska there was, however, little correlation between fertility and type of segregate. An attempt was also made to classify the various individuals according to the number of homozygous and heterozygous factors for the various characters involved. There is some indication in the first cross that with the increase in the number of homozygous factors the number of grains per spikelet is increased,

but the correlations are neither high nor consistent. In the second cross there are no significant correlations between fertility and number of homozygous or heterozygous factors. This method is not entirely satisfactory, however, since many factors might be in a heterozygous condition without resulting in any qualitative differences which can be described and tabulated.

It would appear from these results that segregates combining the desirable characters of the emmer group and the vulgare group can rarely be obtained in the comparatively sterile combinations, but that in the more fertile crosses such individuals might be obtained more frequently. It has previously been found that there is a very high degree of association between the various characters of the emmer and vulgare wheat in hybrid segregates (11). It is found, for instance, that most of the segregates which are rust resistant also resemble the durum or emmer parent in morphological characters and quality of grain. However, several vulgare segregates which were rust resistant were found in about 20,000  $F_2$  and  $F_3$  segregates of crosses between wheats in the emmer and vulgare groups (7). None of these segregates was of economic value, however. By using species which result in relatively fertile segregates the probabilities of obtaining  $F_2$  segregates which would combine the desirable characters of the emmer and vulgare wheats would be greatly increased, although it is not known at the present time how fertile and homozygous these types might be in subsequent generations. It is possible that ultimately all of the intermediate types would be eliminated, although Sevier wheat is an economic variety combining several durum and vulgare characters.

In comparing the genetic behavior of certain characters in the species hybrids it is also desirable to know how these same characters behave in fertile varietal crosses. Such characters as awn length, color of awns, color of grain and pubescence, and in certain cases hardness of grain, have been found to be dependent on Mendelian factor differences in varietal crosses within the vulgare group. It is obvious that certain characters used in species crosses can not readily be analyzed in fertile crosses because the characters are more or less common to the entire group. Such characters, for instance, as size of keel, extremely flinty grain, pithy culms and very long awns are found only in the emmer group and in very few cases are any of these characters absent in any variety within the emmer series. It is true that a few beardless varieties of durum and emmer wheats exist, but these have been derived for the most part, if not entirely, from crosses between members of the emmer group with members of the vulgare group. There is, however, one character which is found in Alaska and in none of the other varieties of the emmer group. This is the branching habit of the spike. An analysis of the behavior of this character was obtained from data on a cross between Alaska  $\times$  Spring emmer. The  $F_1$  was slightly branched although the  $F_1$  plants frequently had some heads which were normal. In  $F_2$  the segregates showed 294 normal to 107 branched. Only six of the  $F_2$  plants were branching to the same extent as the Alaska parent. The degree of branching varied all the way from a reduplication of one or two spikelets to individuals which closely resembled the branched parent. The fact that there is much greater variation in the branched segregates, which are apparently recessive, is not unusual, as in *Chelidonium* doubleness is a recessive character and yet the doubles vary enormously while the singles do not vary at all in regard to petal number. The genetic behavior of this cross is being continued into  $F_3$  for verification of  $F_2$  classification. At any rate, it is evident that the genetic behavior in the varietal cross is entirely different than it is in the species cross. In the cross between Marquis  $\times$  Alaska only five individuals were classed as branched in a population of 152 plants. In  $F_3$  17 families bred true for normal, 89 segregated, and only 4 bred true for the branched condition.

THE RELATION OF CHROMOSOME NUMBER TO MORPHOLOGICAL CHARACTERS IN F<sub>3</sub> AND F<sub>4</sub> SEGREGATES

It has been found that the segregates of emmer X vulgare wheat which resemble the emmer parent have 14 chromosomes, while those which resemble the vulgare parent have 21 chromosomes and that the segregates with an intermediate number of chromosomes tend to become eliminated, due to sterility (11).

In a previous study a number of F<sub>3</sub> segregates were found which had an intermediate chromosome number and others were found which had intermediate head type. In some cases the segregates which were recorded as having intermediate chromosome numbers were of the durum or vulgare type. In one case a segregate was classed as a durum type but had 21 chromosomes. It was considered desirable to determine if the intermediate chromosome numbers and intermediate plant types persisted in the fourth generation, and to obtain additional data in regard to the relation between chromosome number and type of spike. One might also expect a larger proportion of F<sub>3</sub> and F<sub>4</sub> segregates to have a chromosome number of 14 or 21 in Amby X Kubanka than would be the case in the more fertile cross of Marquis X Alaska, where it might be expected that a comparatively large proportion of the segregates would be intermediate in chromosomes number as well as morphological characters. The F<sub>4</sub> segregates of these intermediate types for both Amby X Kubanka and Marquis X Alaska were grown in Maine in 1923. Chromosome counts were obtained with the use of Belling's method (1). It was not always possible to distinguish the univalent chromosomes from the bivalent, so that the numbers of chromosomes of each type could not be exactly determined. The descriptions of the F<sub>3</sub> and F<sub>4</sub> segregated of each cross, the chromosome number where it has been determined and the fertility of the F<sub>4</sub> segregates are shown in Table VI.

TABLE VI.—Type of F<sub>2</sub> and F<sub>3</sub> segregates and chromosome number, grains per spikelet (g) shown for F<sub>4</sub> plants. The numbers and letters correspond to numbers and letters of types of F<sub>2</sub> plants shown in Plates 1 and 2. S=single chromosomes

AMBY X KUBANKA					
No.	Description, 1922, F <sub>3</sub>	Chromosome No.	Description, 1923, F <sub>4</sub>	Chromosome No.	g
	Plate 2				
1	Durum type.....	±16	Durum type.....	14	1.9
2	do.....		do.....	14	1.8
3	do.....		do.....	14	1.9
4	do.....		do.....	14	0
5	do.....	±16	do.....	14	1.9
6	do.....	14+4-6 S	do.....	14	1.1
7	Intermediate.....	14+3-4 S	Intermediate.....	18+2-4 S	1.3
8	Vulgare type.....	18	Vulgare type head—durum awns and grain..	21	2.0
9	Intermediate.....	21	Vulgare type.....	20+1-2 S	1.5
10	do.....	21	do.....	21	2.0
MARQUIS X ALASKA					
	F <sub>3</sub>		F <sub>4</sub>		
	Plate 1				
a	Durum head-vulgare keel.....		Durum head—vulgare keel.....	14	0.8
b	Durum head-soft grain.....		Durum head—soft grain.....	14	2.0
c	do.....		do.....	14	1.6
d	Vulgare head—durum keel.....		Branching head—hard grain.....	18+2-3 S	1.5
e	Vulgare head—durum keel and awns.....		Vulgare head—durum keel and awns.....	18+2-3 S	1.5
f	do.....		Branching head—intermediate keel.....	18+2 S	.5
g	Vulgare head—intermediate keel—durum awns.....		Vulgare head—durum awns, soft grain, intermediate keel.....	19+2-3 S	2.0
h	do.....		Vulgare head—intermediate keel—hard grain.....	19+2-3 S	2.0



In the cross Amby  $\times$  Kubanka several  $F_3$  segregates were classed as the durum or intermediate type but had more than 14 chromosomes. Number 6, for instance, was classed as a durum type in  $F_3$  and had 14 bivalent and 5 or 6 single chromosomes (S), a total of about 20 haploid chromosomes. When these various segregates were carried to  $F_4$  it was found that all of them had 14 chromosomes and were all of the durum type. The fertility varied greatly, ranging from 0 grains per spikelet to individuals as fertile as the parent. Number 7, which was classed as intermediate in  $F_3$  and also had an intermediate number of chromosomes, was intermediate and had an intermediate chromosome number in  $F_4$ . This individual was relatively sterile in  $F_4$ . One vulgare type recorded as having 18 chromosomes in  $F_3$  was found to have 21 chromosomes in  $F_4$ , but had the typical durum awns and the hard grain typical of Kubanka. It was also as fertile as the parent. This case is unusually interesting because apparently two characters peculiar to the durum group are found in a 21 chromosome segregate. Whether or not this individual will be found homozygous and completely fertile in later generations is, of course, questionable. Two  $F_3$  segregates classed as intermediate and with 21 chromosomes were found to segregate vulgare types of spikes in  $F_4$  and had approximately 21 chromosomes each. Pictures of these  $F_4$  segregates are shown in Plate 2 and are arranged in the same order as they are in the Table.

In the cross Marquis  $\times$  Alaska a number of  $F_3$  segregates were selected which appeared to be intermediate and which possessed combinations of durum and vulgare characters. In  $F_4$  certain of these segregates maintained the combination of parental characters and in most cases had an intermediate number of chromosomes, usually with several single lagging chromosomes at the time of the reduction division. One segregate in  $F_4$  had a durum type of head but with the small vulgare type of keel (Plate 1). This individual had 14 chromosomes, but was relatively sterile, as indicated by the number of grains per spikelet. An unusual combination was found with a durum head type but with soft grain. This individual had 14 chromosomes and was practically as fertile as the parent. The other  $F_4$  segregates for which chromosome counts were made were found to have an intermediate chromosome number and ranged in fertility from 0.5 grains per spikelet to 2 grains per spikelet. Number 7, in  $F_4$ , had wrinkled grain, indicating its heterozygous condition and undoubtedly its progeny in  $F_5$  would show various degrees of sterility. It will be of interest to follow these intermediate types into future generations and determine the relation of morphological characters and chromosome numbers.

There are several cases where the characteristics of the two parents are combined in a single individual and this individual may possess the parental chromosome numbers of 14 or 21. These results may indicate that in rare cases segregates can be recovered combining certain of the parental characters. Practical breeding experiments made in an attempt to combine the desirable characters of the emmer wheats with the bread-making qualities and yield of the vulgare wheats indicate, however, that the possibilities of recovering such intermediate types are rare and that the best results can be obtained by selecting the parental varieties within the vulgare group (2, 9, 12, 4, 5, 6).

### CONCLUSIONS

Crosses between wheat species of the emmer group with 14 chromosomes, with members of the vulgare group with 21 chromosomes, result in partially sterile  $F_1$  hybrids, and all degrees of sterility in  $F_2$ . Previous cytological studies of chromosome number and behavior in  $F_1$  and  $F_2$  indicate that gametes and segregates with an intermediate chromosome number tend to be eliminated

through sterility and that the ultimate homozygous fertile segregates will have either 14 or 21 chromosomes (10, 11). Segregates with 14 chromosomes resemble the emmer parent in most respects while segregates with 21 chromosomes possess most of the vulgare characters, indicating that the 7 additional chromosomes determine the differentiating characters of the vulgare wheats.

The elimination of gametes and segregates with an intermediate chromosome number would disturb genetic segregation in case of factors located in the 7 chromosomes contributed by the vulgare parent which do not pair in the reduction division of  $F_1$  crosses between emmer and vulgare wheats. Normal Mendelian segregation should usually occur with those factors located in the 14 emmer and vulgare chromosomes which pair in  $F_1$  hybrids. Thus one might expect normal Mendelian segregation of characters common to the two groups of wheat species and aberrant segregation of characters which distinguish the two groups. Of the characters analyzed in both  $F_2$  and  $F_3$  of such species crosses, the presence or absence of awns, yellow or black awns, and red and white grain are all characters found in either the emmer group or the vulgare group although black awns are rare in vulgare wheats (3, 9). The segregation of awn length into so-called awnless (tip-awned) and awned is clearly dependent on a single factor difference and a normal Mendelian segregation is obtained. The segregation of awn color is complicated by the effect of environmental factors but  $F_3$  classification indicates a simple Mendelian ratio. The number of segregates with white grain in the cross of Marquis  $\times$  Alaska would indicate the presence of two factors for red color although the number of segregating individuals is greater than expected.

On the other hand, the characters which distinguish the emmer and vulgare groups apparently do not segregate in a Mendelian fashion, as indicated by the data presented in Tables II and III. Most varieties of the emmer group have very hard flinty kernels, a large sharp keel on the outer glume and some varieties have branched spikes. These characters are found in no vulgare variety. Since these characters are common to all of the varieties in the emmer group it is impossible to determine how they are inherited in fertile varietal crosses. There is, however, one exception in the case of Alaska. Alaska has the branched spike while the durum and emmer wheats have a normal spike. The fact that the cross of Alaska  $\times$  Emmer resulted in an apparently simple ratio of normal and branched or at least the genetic behavior is entirely different than in the species hybrids. The nonMendelian segregation of these typical emmer characters in the species hybrids suggests that they may be dependent on the presence or absence of factors located in the extra seven vulgare chromosomes which do not pair in crosses with emmer wheat. Since these seven chromosomes are irregularly distributed and certain classes are apparently eliminated due to sterility, a normal Mendelian segregation would not be expected.

Certain crosses between members of the vulgare group and members of the emmer group are more fertile than others as indicated in Table I. In the more fertile crosses segregates may possess certain combinations of parental characters and intermediate chromosome numbers while in the more sterile crosses the intermediate types would be rapidly eliminated and the surviving segregates would have either 14 or 21 chromosomes and would resemble the parental species. Thus there should be considerable correlation or association of the characters contributed by each parent in the more sterile hybrids while in the more fertile crosses there would be less tendency for the characters of each parent to be associated. This is actually the case, as indicated by the correlations presented in Table V. These results would indicate that combinations of the characters of the emmer and vulgare wheats can be obtained more frequently in the more fertile combinations. However, many plant breeders have attempted to com-

bine the drought and disease resistance of the emmer species with the high quality of the vulgare wheats without successful results.

In general, the intermediate types of segregates are more sterile than segregates resembling one parent or the other (Tables IV and V). Individuals resembling one economic species of wheat are more fertile than those which can not be so classed.

There is a greater proportion of intermediate types in the more fertile species hybrids, which may indicate that more of the segregates can function with an intermediate chromosome number in such crosses. A cytological examination of  $F_4$  segregates shows that the intermediate types usually have an intermediate chromosome number but some of the segregates with 14 and 21 chromosomes also possess characteristics of both parents. The presence of some of the typical vulgare characters in 14 chromosome segregates and the occasional recovery of emmer characters in 21 chromosome segregates suggest that the chromosomes which carry the factors for the distinguishing vulgare characters may in some cases at least pair with certain of the emmer chromosomes in crosses between members of the two groups. The combinations of typical emmer and vulgare characters in certain segregates indicates that the differentiating vulgare characters are determined by factors in individual chromosomes and not by the combined effect of the additional seven chromosomes.

Although in the more fertile crosses there are many segregates with intermediate chromosome numbers it is questionable if such segregates can persist in a homozygous fertile condition. Certain combinations of emmer and vulgare characters do occur in segregates with 14 or 21 chromosomes and it is probable that a small proportion of segregates can be obtained which will combine certain characters of the two groups of species, especially in the more fertile combinations. From a practical standpoint, however, it would probably be more feasible to combine disease resistance and yield and quality of grain by selecting the parents within the vulgare group. It is perhaps significant that crosses or vulgare varieties are apparently the only ones which have resulted in new varieties of economic importance.

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## PLATE 1

### Parental varieties used for crossing

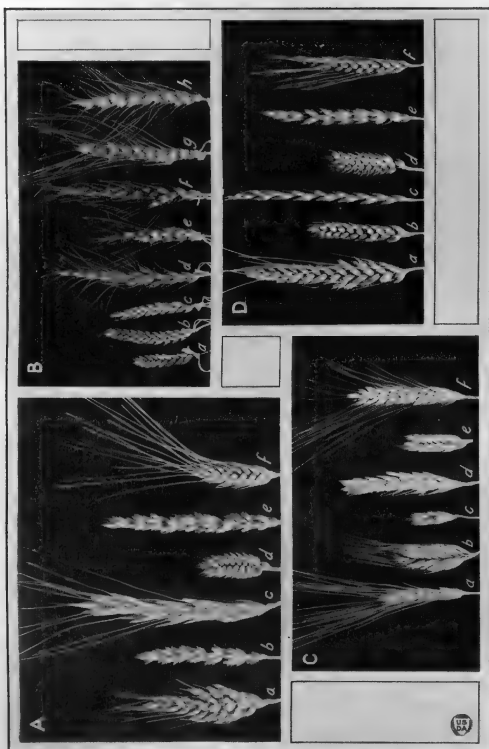
- A, *a*.—*Triticum turgidum* (Alaska).  
*b*.—*T. vulgare* (Amby).  
*c*.—*T. plonicum* (Polish).  
*d*.—*T. compactum* (Washington Hybrid 143).  
*e*.—*T. vulgare* (Marquis).  
*f*.—*T. durum* (Kubanka).

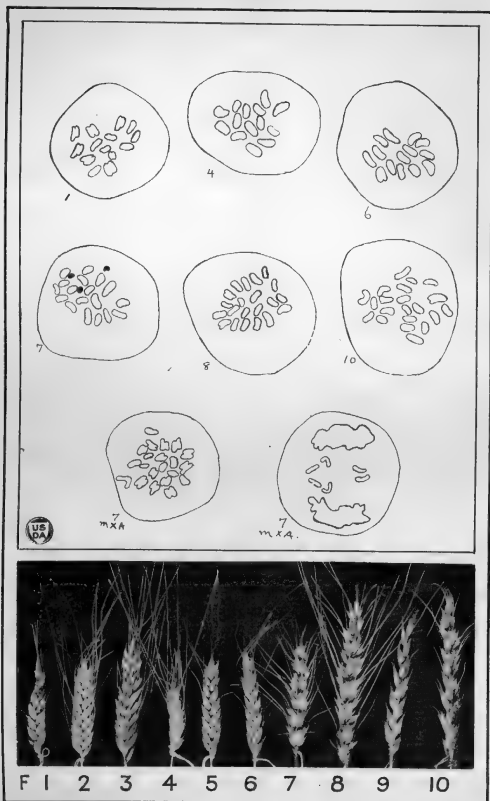
### Marquis $\times$ Alaska $F_4$

B. *a-h*.—Various types of segregates resulting from the most intermediate types of  $F_3$  segregates. The numbers correspond to the numbers in Table VI, where the description, chromosome number, and sterility of each plant is presented.

D. *a-f*.—Various types of  $F_2$  segregates from the cross Marquis  $\times$  Alaska. In addition to parental types individuals are recovered which resemble *T. Spelta* and *T. durum*.

C. *a-f*.—Types of segregates from the  $F_2$  of the cross Hybrid 143  $\times$  Polish.





## PLATE 2

Figures 1, 4, 6, 7, 8, 10 are from camera lucida drawings of  $F_4$  segregates of Amby  $\times$  Kubanka and correspond to the numbers of the segregates shown below. These figures are from pollen mother cells at the time of the first reduction division.

Figures numbered 7 (M  $\times$  A) are from segregate *g* of Marquis  $\times$  Alaska shown in Plate 1. The side view of the heterotypic division shows the three lagging chromosomes soon after they have divided.

F. 1-10.—Segregates of Amby  $\times$  Kubanka  $F_4$ . The numbers correspond to the chromosomes figures shown above and to the numbers shown in Table VI. The description, chromosome number, and degree of sterility of each of the segregates pictured are shown in Table VI.



# DETERMINATION OF TEMPERATURES FATAL TO THE POWDER-POST BEETLE, *LYCTUS PLANICOLLIS* LECONTE, BY STEAMING INFESTED ASH AND OAK LUMBER IN A KILN<sup>1</sup>

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## INTRODUCTION

Previous preliminary experiments conducted to determine temperatures fatal to *Lyctus* "powder-post" beetles in the dry kiln have indicated that it is necessary to attain a high temperature for a short period, that is, where the humidity does not reach the saturation point, a temperature of 180° F. must be maintained for at least one-half hour after the ordinary kiln-drying operation.<sup>2</sup> There are objections to subjecting wood to such high temperatures, especially where great structural strength is demanded in its subsequent use, as in the construction of airplanes, for it is likely to weaken the wood fibers as well as to cause discoloration. With these disadvantages in mind, and the knowledge that it is more economical to steam infested lumber in a kiln at a low temperature for a short period than at a high one after the longer period of kiln drying, it was decided to conduct some experiments by subjecting lumber to live steam at lower temperatures for short periods with a saturated atmosphere, to obtain the range of temperatures fatal to the powder-post beetles. Through the courtesy of officers of the United States Navy Department, Bureau of Construction and Repair, and the Naval Aircraft Factory at the navy yard, League Island, Philadelphia, Pa., a series of cooperative experiments along this line was conducted in November and December, 1923, and in January, 1924.<sup>3</sup>

## MATERIAL TREATED

The material used in these experiments consisted of infested ash and oak lumber. The former had been piled for nearly 10 years in the open where it was exposed to attack by the southern powder-post beetle, *Lyctus planicollis* Le Conte. The larvæ of this beetle had practically ruined the entire sapwood, rendering it unfit for any use other than fuel. All of the stock was of high-grade material. The ash lumber, being the more seriously damaged and the more infested with insects, was the wood principally used in the experiments.

## THE KILN

The kiln used is of the water-spray type and its dimensions are approximately 60 by 14 by 14 feet. Live steam is admitted into it through two pipes, 1½ inches in diameter, running lengthwise through the center. Along the upper side of these pipes, at intervals of 12 inches, there are holes one-fourth of an inch in

<sup>1</sup> Received for publication Apr. 22, 1924—issued Nov., 1924.

<sup>2</sup> SNYDER, T. E. HIGH TEMPERATURES FOR THE CONTROL OF *LYCTUS* "POWDER-POST" BEETLES. *Jour. Forestry* 21: 810-814. 1923.

<sup>3</sup> Special acknowledgment should be made to Capt. G. N. Rock, Bureau of Construction and Repair, Navy Department, Washington, D. C., A. N. Miller, mill foreman, Naval Aircraft Factory, Philadelphia, Pa., and C. C. Shackford, Naval Aircraft Factory, whose hearty cooperation have made these tests possible.

diameter through which the steam is allowed to escape into the kiln for a certain length of time, six hours for every inch of thickness of the lumber being formerly the period of steaming. Then dry heat is applied and the hot air rises through the lumber, cools, and settles toward the bottom of the kiln. The circulation is completed by air currents set in motion by sprays of water coming from jets about 18 inches apart, directed downward, on pipes along the sides of the kiln. These jets of water help to regulate the humidity. After the cool air reaches the bottom it passes through the pipes again where it is brought to a higher temperature.

The temperatures in the following experiments were governed by a Taylor recording thermometer. This was controlled by placing a maximum thermometer in the kiln on the samples undergoing treatment. The relative humidity, as previously stated, was constant at 100. All temperatures were recorded in degrees Fahrenheit.

### PREPARATION OF SAMPLES

The infested lumber was placed in a kiln which was kept at a low temperature (100° F.) for several days to determine if the powder-post beetles were still in the wood.<sup>4</sup> An examination of the material at the end of this time showed that it was still well infested. The presence of active larvæ was shown by piles of boring dust, or powderlike frass, found on and about the pieces of lumber (Pl. 1, A).

The samples were prepared by sawing the ash lumber into boards 1 by 12 by 18 inches and into planks 2 by 12 by 18 inches, making a total of 28 pieces of the former and 69 of the latter, and the oak into 4 pieces, each 1½ by 12 by 18 inches in size. The samples were divided into three lots as follows:

Lot I. Ash, 7 pieces 1 inch thick and 14 pieces 2 inches thick; oak, none.

Lot II. Ash, 12 pieces 1 inch thick and 6 pieces 2 inches thick; oak, 4 pieces 1½ inches thick.

Lot III. Ash, 9 pieces 1 inch thick and 20 pieces 2 inches thick; oak, none.

These boards 1 and 1½ inches thick and planks 2 inches thick were subjected to temperatures ranging from 120° to 160° F. and for different lengths of time. On account of the danger of running the thermostat at a temperature above 160° F., the maximum at which it operates, and because previous experiments by C. C. Shackford had proved that this temperature is fatal to the powder-post beetles, no attempt was made to exceed it.

The pieces were marked with their lot numbers and each was given an individual number. The locations of piles of frass on the samples were marked with blue crayon (Pl. 1, A, B). After the wood was removed from the kiln, these places were watched for signs of activity. Lot I was left in the kiln overnight at a higher temperature than that to which the material had been subjected previously. This caused increased activity and additional piles of frass were noted (Pl. 1, A), proving that the samples were more heavily infested than previously indicated by the piles of frass on the outside. The proportion of sapwood and heartwood was also noted, as well as the depth of the larvæ from the nearest surface. C. C. Shackford ran the kiln and assisted R. A. St. George with the work of inspection.

### PROCEDURE

Each lot was treated by holding it at a certain temperature for a definite period of time before changing to a higher temperature, and removing pieces at every 10° interval. At least one piece of each lot was examined by chopping into the

<sup>4</sup> Heating the material in this way was necessary because insects are normally inactive at the low temperatures of the fall and winter months.

sample, noting the condition of the larvæ and placing them in vials for future observation. Copper tags (Pl. 1, A, B) furnishing data as to the treatment were placed on the remaining pieces, which were put in a heated kiln for further observation. Photographs were taken of the samples before and after treatment. After the treatment C. C. Shackford examined the material from time to time and noted its condition. A final examination was made four weeks later. These tests were conducted in practically an empty kiln. When the kiln is full of lumber, time should be allowed for all parts of the wood to reach kiln temperature, at which it should be held for the length of time found to be fatal to the insects.

LOT I

Because it had been previously determined that dry heat at 120° F. was not fatal to the powder-post beetle, indicating that a kiln with a saturated atmosphere would require a higher temperature or a longer period of time, no sample pieces in Lot I were removed until after an exposure of one and one-half hours to a temperature of 130° F.

The initial treatment of Lot I was similar to that used in commercial operations for drying lumber. The lot was placed in the kiln at a low temperature, which was gradually raised, and the lumber left in the kiln over night. This was done to make sure that all parts of the samples were at kiln temperature when the temperature of the wood reached the initial point of the experiment. At 3 p. m. December 11 the samples were placed in the kiln at a temperature of 70° F. By 4 p. m. the temperature had been raised to 100° F., where it remained until 3 a. m. December 12. At 8.15 a. m. it reached 110° F. and at 9.40 a. m. 130° F., where it was held for one and one-half hours. Upon removal of the first pieces the temperature was raised 10 degrees and held there for the same length of time, this operation being repeated until the last lot was removed from the kiln at 160° F. The maximum thermometer showed that the indication of the recording thermometer was 4° too low during the work on this lot.

TABLE I.—Experiments to determine temperatures fatal to *Lyctus planicollis* by steaming infested ash lumber in a kiln. Lot I

Sample No.	Size	Temperature and time held		Time held at accumulated temperature starting at 130° F.	Humidity		Proportion of sap-wood	Number of piles of frass noted before steaming	Larvæ chopped out immediately after steaming—number and condition	Final examination, Jan. 10, 4 weeks after steaming. Piles of frass noted; larvæ chopped out where results were doubtful <sup>a</sup>
		°F.	Hours.		Per cent.	Per cent.				
1	1×12×18..	130	1½	1½	100	25	(b)	-----	-----	No signs of activity.
2	1×12×18..	140	1½	3	100	95	15	-----	-----	Do.
3	1×12×18..	140	1½	3	100	95	(b)	3, darkened..	-----	Do.
4	1×12×18..	150	1½	4½	100	90	1	-----	-----	Do.
5	1×12×18..	150	1½	4½	100	95	3	c-----	-----	Do.
6	1×12×18..	160	1½	6	100	100	(b)	-----	-----	Do.
7	1×12×18..	160	1½	6	100	100	(b)	-----	-----	Do.
8	2×12×18..	130	1½	1½	100	25	1	1, white	-----	Do.
9	2×12×18..	130	1½	1½	100	95	15	12, white; 9, darkened.	-----	Do.
10	2×12×18..	140	1½	3	100	20	(b)	c-----	-----	Do.
11	2×12×18..	140	1½	3	100	10	1	-----	-----	Do.
12	2×12×18..	140	1½	3	100	25	3	-----	-----	Do.
13	2×12×18..	140	1½	3	100	20	1	-----	-----	Do.
14	2×12×18..	150	2½	4½	100	25	3	3 larvæ (1 white).	-----	Do.
15	2×12×18..	150	2½	4½	100	15	1	-----	-----	Do.
16	2×12×18..	150	2½	4½	100	15	1	-----	-----	Do.
17	2×12×18..	150	2½	4½	100	20	1	-----	-----	Do.
18	2×12×18..	160	2½	6	100	5	(b)	-----	-----	Do.
19	2×12×18..	160	2½	6	100	5	(b)	-----	-----	Do.
20	2×12×18..	160	2½	6	100	20	7	-----	-----	Do.
21	2×12×18..	160	2½	6	100	10	1	c-----	-----	Do.

<sup>a</sup> Examined by C. C. Shackford December 15, 17, and 21, and January 3; checked by R. A. St. George on final examination.  
<sup>b</sup> No piles of frass noted, but all samples infested.  
<sup>c</sup> Examined; none found.

An examination of the larvæ chopped out on December 17 showed that they were all apparently dead, although some were still white and soft. The pieces that were tagged and placed in the kiln for future observation were examined December 14 and did not show any signs of larval activity. A final examination of this lot was made January 10, which verified the foregoing preliminary results (Pl. 1, B).

LOT II

Lot II was held for a longer time at every 10° interval than Lot I. It was not deemed necessary to subject any samples to 160° F. The four pieces of oak were included in this lot. All of the samples were placed in the kiln and their temperature raised at once to the initial temperature without previously bringing all parts of the wood to kiln temperature, as was done with Lot I. At 8.30 a. m., December 13, the lot was placed in the kiln at 100° F. From 8.30 to 9 a. m. the temperature advanced to 130°, where it was held for two and one-half hours, or one hour longer than in the case of the previous lot. From 130° onward, pieces were removed at every 10° interval until the temperature reached 150°. The maximum thermometer showed that during the work on Lot II the indication of the recording thermometer was 2° too low.

TABLE II.—*Experiments to determine temperatures fatal to Lyctus planicollis by steaming infested ash and oak lumber in a kiln. Lot II*

Sample No. <sup>a</sup>	Size	Temperature and time held		Time held at accumulated temperature starting at 130° F.	Humidity	Proportion of sap-wood	Number of piles of frass noted before steaming	Larvæ chopped out immediately after steaming—number and condition	Final examination, Jan. 10, 4 weeks after steaming. Piles of frass noted; larvæ chopped out where results were doubtful <sup>b</sup>
		° F.	Hours.						
1	Inches. 1×12×18	130	2½	Hours. 2½	Per cent. 100	Per cent. 25	(c)	-----	No signs of activity.
2	1×12×18	130	2½	2½	100	25	2	-----	Do.
3	1×12×18	130	2½	2½	100	100	(c)	-----	Do.
4	1×12×18	130	2½	2½	100	100	(c)	-----	Do.
5	1×12×18	140	2½	5	100	100	2	3 larvæ (1 white).	Do.
6	1×12×18	140	2½	5	100	100	2	-----	Do.
7	1×12×18	140	2½	5	100	100	2	-----	Do.
8	1×12×18	140	2½	5	100	100	1	-----	Do.
9	1×12×18	150	2½	7½	100	100	1	-----	Do.
10	1×12×18	150	2½	7½	100	100	(c)	-----	Do.
11	1×12×18	150	2½	7½	100	40	1	-----	Do.
12	1×12×18	150	2½	7½	100	100	(c)	-----	Do.
13	2×12×18	130	2½	2½	100	30	2	-----	Do.
14	2×12×18	130	2½	2½	100	30	2	-----	Do.
15	2×12×18	140	2½	5	100	25	3	-----	Do.
16	2×12×18	140	2½	5	100	25	2	-----	Do.
17	2×12×18	150	2½	7½	100	30	2	-----	Do.
18	2×12×18	150	2½	7½	100	10	5	-----	Do.
19	1½×12×18	130	2½	2½	100	3	(c)	-----	Do.
20	1½×12×18	140	2½	5	100	5	(c)	-----	Do.
21	1½×12×18	150	2½	7½	100	3	(c)	-----	Do.
22	1½×12×18	150	2½	7½	100	3	(c)	-----	Do.

<sup>a</sup> Nos. 1-18 are ash; Nos. 19-22 are oak.  
<sup>b</sup> Examined by C. C. Shackford December 15, 17, and 21 and January 3; checked by R. A. St. George on final examination.  
<sup>c</sup> No piles of frass noted, but all samples infested.

An examination of the larvæ chopped out on December 17 showed that they were all dead, although some were still white and soft. The tagged pieces were examined with those of Lot I and the results were found to be the same. A final examination of this lot on January 10 showed that all the insects had been killed and the damage checked (Pl. 1, B).

## LOT III

Lot III was placed in the kiln at 8.30 a. m., December 14, at a temperature of 90° F., which was raised to the initial point, 120° F., by 9 a. m., and held there one-half hour. Pieces were removed at 10° intervals up to 160° F. Samples were taken out at 120° and 160° F., as well as at the intermediate intervals, because samples in Lots I and II subjected to these temperatures apparently did not contain any active larvæ. It was decided to make removals every half hour to determine when the larvæ ceased to be active. The first two removals at 120° and 130° F. probably did not allow the samples to remain in the kiln long enough for all parts of the wood to reach the kiln temperature. The maximum thermometer indicated that the recording thermometer read 5° too low at 120° F., 2° too low at 130° F., 3° too low at 140° F., 2° too low at 150° F., and 1° too low at 160° F.

TABLE III.—*Experiments to determine temperatures fatal to Lyctus planicollis by steaming infested ash lumber in a kiln. Lot III*

Sample No.	Size	Temperature and time held		Time held at accumulated temperature starting at 130° F.	Humidity	Proportion of sap-wood	Number of piles of frass noted before steaming	Larvæ chopped out immediately after steaming—number and condition	Final examination, Jan. 10, 4 weeks after steaming. Piles of frass noted; larvæ chopped out where results were doubtful <sup>a</sup>
		° F.	Hours.						
1	2×12×18	160	½	2½	100	20	3		No signs of activity.
2	2×12×18	160	½	2½	100	85	(b)		Do.
3	2×12×18	160	½	2½	100	15	1		Do.
4	2×12×18	160	½	2½	100	20	3		Do.
5	2×12×18	150	½	2	100	15	(b)		Do.
6	2×12×18	150	½	2	100	15	3		Do.
7	2×12×18	150	½	2	100	15	2		Do.
8	2×12×18	150	½	2	100	15	2		Do.
9	2×12×18	140	½	1½	100	25	(b)		Do.
10	2×12×18	140	½	1½	100	15	(b)		2 piles of frass noted Dec. 21. <sup>a</sup>
11	2×12×18	140	½	1½	100	20	2		No piles of frass noted Dec. 21; 6 on Jan. 10. <sup>a</sup>
12	2×12×18	140	½	1½	100	15	(b)		No signs of activity.
13	2×12×18	130	½	1	100	15	(b)		Do.
14	2×12×18	130	½	1	100	10	(b)		4 piles of frass noted Jan. 3. <sup>a</sup>
15	2×12×18	130	½	1	100	20	2		5 piles of frass noted Jan. 3. <sup>a</sup>
16	2×12×18	130	½	1	100	20	(b)	Clerid larva alive; white Lyctus.	36 piles of frass noted Dec. 15. <sup>a</sup>
17	2×12×18	120	½	½	100	20	(b)		17 piles of frass noted Dec. 17. <sup>a</sup>
18	2×12×18	120	½	½	100	20	(b)		2 piles of frass noted Dec. 21. <sup>a</sup>
19	2×12×18	120	½	½	100	25	1		11 piles of frass noted Dec. 15. <sup>a</sup>
20	2×12×18	120	½	½	100	95	10	2 Lyctus, 1 clerid alive.	34 piles of frass noted Dec. 15. <sup>a</sup>
21	1×12×18	160	½	2½	100	100	5		No signs of activity.
22	1×12×18	160	½	2½	100	15	(b)		Do.
23	1×12×18	150	½	2	100	25	4		Do.
24	1×12×18	150	½	2	100	20	1		Do.
25	1×12×18	140	½	1½	100	15	(b)		Do.
26	1×12×18	140	½	1½	100	15	(b)		Do.
27	1×12×18	130	½	1	100	15	(b)		Do.
28	1×12×18	130	½	1	100	15	(b)		Do.
29	1×12×18	120	½	½	100	5	(b)		4 piles of frass noted Dec. 29. <sup>a</sup>

<sup>a</sup> Reported by C. C. Shackford and checked on final examination by R. A. St. George.

<sup>b</sup> No piles of frass noted, but all samples infested.

An examination of the larvæ chopped out on December 17 showed that temperatures of 120° and 130° F., when held for only one-half hour, were not fatal to the insects. Living larvæ of the powder-post beetle were obtained from these samples. In addition, immature and mature stages of a beneficial insect known to be predacious on the powder-post beetle were also found alive and in considerable numbers. There were not enough present, unfortunately, to exterminate the injurious forms.

A further examination on December 21 verified these results and showed a revival of the larvæ in samples subjected to temperatures varying from 120° to 140° F. when held there for only one-half hour (Pl. 1, A). Other samples in these lots showed that the larvæ were affected and did not recover until January 3.

A final examination was made on January 10, and the above-recorded results verified. Larvæ probably revived in the samples subjected to temperatures up to 140° F. because all parts of the wood had not reached kiln temperature when removed, or, if so, were not held at that temperature long enough to cause the death of the insects. The samples held at 140° F. had an accumulation of temperatures for one and one-half hours.

It will be noted that samples of Lot I were exposed to temperatures gradually raised to 130° F., and were kept at that temperature for 1½ hours. This treatment was fatal to the powder-post beetles because the wood had remained in the kiln over night at temperatures ranging from 100° to 110° F., and all parts of the samples had reached kiln temperature. Under such conditions the subsequent treatment proved fatal to the beetles.

Results obtained from treating Lot II show that placing samples in the kiln, raising the temperature at once to the initial temperature of 130° F., and holding it there for 2½ hours, is effective in killing any powder-post beetles in the wood.

### CONCLUSIONS

Results obtained from the foregoing experiments may be briefly summarized as follows:

1. Temperatures below 130° F. are not fatal to the powder-post beetle *Lyctus planicollis* Lec. when the temperature of infested ash and oak lumber is raised to these temperatures in a kiln by the use of live steam and are held there for only one-half hour, if all parts of the wood have not previously been brought to kiln-drying temperature.

2. Temperatures of 130° F. and upward, maintained for 1½ hours, or longer, are fatal to these insects if all parts of the wood infested by them have at the beginning of the exposure to these temperatures been brought to the minimum temperature of 130° F.

3. The standard kiln-drying schedule for ash and oak, to be used for aircraft stock, in a kiln operated by live steam will prove fatal to the powder-post beetle and will check all damage that is being done in any infested material.

## PLATE 1

### *Lyctus planicollis*

A.—Samples of infested wood showing evidences of resumption of larval activity 15 hours after treatment by steaming in a kiln. The wood was held in the kiln one-half hour at a temperature of 120° F. Circles indicate where activity was noted before treatment. (Lot III, Nos. 16 and 20.)

B.—Samples showing absence of resumption of larval activity four weeks after treatment. Held in kiln 2½ hours at a temperature of 130° F. (Lot I, No. 14.)





## BACTERIAL BLIGHT OF RYE <sup>1</sup>

By C. S. REDDY, *Pathologist*, JAMES GODKIN, *formerly Assistant Pathologist*, and A. G. JOHNSON, *Pathologist, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture*

Evidently there is more than one bacterial disease of rye (*Secale cereale*). In an earlier publication by Jones, Johnson, and Reddy <sup>2</sup>, a bacterial disease of rye, occurring near Madison, Wis., was referred to, the organism of which was pathogenic not only on rye but also on wheat, spelt, and barley. Both in pathogenicity and in cultural characters the organism was very similar to if not identical with the wheat organism which has been described by Smith, Jones, and Reddy <sup>3</sup> as *Bacterium translucens* var. *undulosum* S. J. & R.

In 1921 a bacterial disease of rye was observed by one of the writers (Reddy) near Bloomington, Ill., where it occurred on the leaves of Rosen (winter) rye. The leaf symptoms of the disease at Bloomington were identical with those previously observed on rye in the vicinity of Madison, Wis. In both cases, blotch-like and stripe-like water-soaked lesions (Pl. 1) were noted, very similar to those described by Jones, Johnson, and Reddy <sup>4</sup> for the bacterial blight of barley. The rather conspicuous exudate which occurs on barley and wheat has also been noted on rye. •

Isolations made from the Bloomington collections yielded a yellow organism to all appearances identical with the rye organism previously referred to. In inoculations on rye, wheat, barley, and oats this new rye organism proved to be pathogenic only on rye. In all of these inoculation experiments the spray method was employed as previously described for the barley organism.

At Bloomington, Ill., in 1921, two inoculation experiments were conducted with the new rye organism. In both experiments rye and Marquis (spring) wheat seedlings about 4 inches high, growing in rows in the field, were inoculated. In each case an ample number of plants were left uninoculated as controls. In both of these experiments typical bacterial lesions resulted on the rye, while the wheat seedlings and all of the controls remained free from bacterial infection. The typical rye organism was recovered from the rye lesions, and it proved pathogenic in subsequent inoculation experiments.

At Madison, Wis., in 1921 and 1922, two inoculation experiments were conducted. In each, both the new rye organism and the barley organism, *Bacterium translucens*, were used as inocula. In both experiments, parallel inoculations with the two organisms were made in the greenhouse on rye, wheat, barley, and oat plants about 4 inches high. In both series the new rye organism produced typical bacterial lesions only on rye and the barley organism, *B. translucens*, infected only barley as usual. All the uninoculated control plants remained free from bacterial infection.

The morphological characters of the new rye organism were found to be identical with those of *Bacterium translucens* as described by Jones, Johnson, and Reddy.<sup>4</sup>

<sup>1</sup> Received for publication April 19, 1924—issued Nov., 1924. These investigations were conducted in cooperation with the Wisconsin Agricultural Experiment Station, Madison, Wis., and the Funk Bros. Seed Co., Bloomington Ill.

<sup>2</sup> JONES, L. R., JOHNSON, A. G., and REDDY, C. S. BACTERIAL BLIGHTS OF BARLEY AND CERTAIN OTHER CEREALS. *Science* 44: 432-433, 1916.

<sup>3</sup> SMITH, E. F., JONES, L. R., and REDDY, C. S. THE BLACK CHAFF OF WHEAT. *Science* 50: 48, 1919.

<sup>4</sup> JONES, L. R., JOHNSON, A. G., and REDDY, C. S. BACTERIAL BLIGHT OF BARLEY. *Jour. Agr. Research* 11: 625-644, illus. 1917.

The cultural characters were studied in direct comparison with those of *Bacterium translucens*, that is, the two organisms were run in parallel series. In all of these studies of cultural characters the two organisms were found to be identical. The results were the same as given for the barley organism, *B. translucens*, except in one detail as follows: In the case of action on starch, it was previously reported for the barley organism, *B. translucens*, that there was "no evidence of diastatic action on potato starch \* \* \*." In the present investigations, using the more delicate methods described by Conn et al.,<sup>5</sup> it was found that both the barley organisms, *B. translucens*, and the new rye organism produced a very feeble diastatic action on starch noticeable for approximately half an hour after the iodine solution had been applied. After that time it was difficult to detect any halo about the colonies. There was possibly a slight dextrine reaction.

In addition, certain special studies were made on indicator media. Saccharine media containing brom cresol purple and cresol red, respectively, were prepared according to the recommendations of Conn et al.<sup>5</sup>

The new rye organism and the barley organism, *Bacterium translucens*, were used in parallel series. Eight agar slants of each medium were inoculated with each organism respectively, and four slants of each medium were left uninoculated. Both organisms changed the color of each medium to the same degree in the same length of time. Alkaline reactions were produced in all of these media as follows: Pronounced in those containing lactose, maltose, mannitol, or glycerine; less pronounced in those containing dextrose; and still less pronounced in those containing saccharose.

On the basis of these studies it is evident that the new rye organism is identical with *B. translucens* and the previously described variety, *B. translucens undulosum*, both in morphological characters and in physiological characters in artificial culture media but differs from both in pathogenicity. Hence, the new rye organism is here designated a new variety of *B. translucens* as follows:

***Bacterium translucens secalis* n. var.**

In morphology and in artificial culture this variety is identical with *Bacterium translucens* and *B. translucens undulosum*.

*Bacterium translucens* infects only barley.

*Bacterium translucens undulosum* infects wheat, barley, rye, and spelt.

*Bacterium translucens secalis* infects only rye.

Type locality: Bloomington, Ill.

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<sup>5</sup> CONN, H. J., and others. REPORT OF THE COMMITTEE ON THE DESCRIPTIVE CHART FOR 1919. *JOHNS BACT.* 5: 127-143; 315-319. 1920.

PLATE 1.

Bacterial lesions from natural infections on leaf-blades of rye. Specimens collected near Bloomington, Ill., in 1921.



# NON-INHERITANCE OF TERMINAL BUD ABORTION IN PIMA COTTON<sup>1</sup>

By THOMAS H. KEARNEY

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The normal development of the cotton plant is often interfered with, and various types of malformation result. One of the most common and characteristic of these teratological states is associated with arrested development or abortion of the terminal bud of the axis. The frequency of this phenomenon is indicated by the fact that of 1,109 plants of Pima cotton under observation in 1918, 44, or approximately 4 per cent, had the terminal bud aborted. In a majority of cases the injury occurs during the seedling stage, 35 of the 44 individuals having shown abortion at the time the cotton was thinned. Yet the terminal bud may be lost at any time during the growth of the plant. An individual in which the injury occurred at a comparatively late stage of development is shown in Plate 1, with an adjacent, uninjured plant for comparison.

The cause of abortion of the terminal bud is not known, although the injury is frequently associated with leaf-cut or tomosis,<sup>2</sup> and indications of injury by the larvae of an insect were observed in one case. If it occurs early in the life of the plant, one of the vegetative branches produced at low nodes of the axis may assume an erect position, simulating so closely a true axis that close observation is required to determine that the original growing point has been destroyed or arrested. If abortion takes place in a later stage of development, as in the individual shown in Plate 1, the fruiting branches just below the point of injury become elongated and often branched, with a tendency to assume a more nearly erect position and to produce abnormally large leaves of coarse texture. The same phenomenon may be induced by artificially "topping" the plant.

As in all such cases, it is important to know whether we are dealing with a heritable character. If this malformation is inherited, aborted individuals should be removed in roguing seed-increase fields, for they are nearly always less fruitful than normal plants. In an endeavor to solve this problem, 12 aborted individuals of the Pima variety of Egyptian cotton, as well as 12 normal individuals to serve as controls, were selected at the United States Field Station, Sacaton, Ariz. The control for each aborted plant was either the next plant in the row or the nearest normal plant that could be found. Each aborted plant and its control were given the same number. Flowers on all of the aborted and normal individuals were bagged to prevent cross-pollination and from the resulting self-fertilized seed progenies of the 24 plants were grown the following year. Each pair of progenies representing a normal and an aborted plant were located in the same portion of the field, so as to reduce to a minimum the influence of soil heterogeneity.

<sup>1</sup> Received for publication April 9, 1924—issued Nov., 1924.

<sup>2</sup> COOK, O. F. LEAF-CUT, OR TOMOSIS, A DISORDER OF COTTON SEEDLINGS. U. S. Dept. Agr. Bur. Plant Indus. Circ. 120: 29-34, illus. 1913.

Table I gives for each progeny the total number of plants before thinning, and the number and percentage of individuals of which the terminal bud was aborted at some stage of development. The data also are combined for all progenies of aborted individuals and all progenies of normal individuals, taken as one array in each case.

TABLE I.—*Numbers and percentages of aborted plants in progenies of aborted and normal individuals of Pima cotton*

Progeny of plant No.	Progenies of aborted plants			Progeny of plant No.	Progenies of normal plants		
	Total in- dividuals	Number aborted	Percentage aborted		Total in- dividuals	Number aborted	Percentage aborted
1.....	43	2	4.6	1.....	37	6	16.2
2.....	34	1	2.9	2.....	43	5	11.6
3.....	44	2	4.5	3.....	48	2	4.2
4.....	38	2	5.3	4.....	43	1	2.3
5.....	47	1	2.1	5.....	53	1	1.9
6.....	49	3	6.1	6.....	45	1	2.2
7.....	55	0	0	7.....	65	4	6.2
8.....	49	1	2.0	8.....	41	3	7.3
9.....	39	1	2.6	9.....	50	0	0
10.....	35	2	5.7	10.....	51	1	2.0
11.....	61	0	0	11.....	45	0	0
12.....	46	3	6.5	12.....	48	2	4.2
Total.....	540	18	3.33±0.52	Total.....	569	26	4.56±.059

When all progenies of aborted and of normal plants, respectively, are considered as one array, the latter give a slightly higher percentage of aborted individuals, but the difference is not significant, being only 1.6 times its probable error. It is concluded that abortion of the terminal bud in this material is not an inherited character.

## PLATE 1

A.—At the right, a plant of Pima cotton with terminal bud of the main stalk aborted; at the left, a normal plant in which the development of the axis has not been interrupted.

B.—The same plants with most of the leaves removed.





# RELATIVE RESISTANCE OF THE RICE WEEVIL, *SITOPHILUS ORYZA* L., AND THE GRANARY WEEVIL, *S. GRANARIUS* L., TO HIGH AND LOW TEMPERATURES<sup>1</sup>

By E. A. BACK, *Entomologist in Charge*, and R. T. COTTON, *Entomologist, Stored Product Insect Investigations, Bureau of Entomology, United States Department of Agriculture*

During several years of study on the grain weevils the writers have observed that the rice weevil, *Sitophilus oryza* L., and the granary weevil, *S. granarius* L., although belonging to the same genus and closely resembling each other in size, structure, and habits, differ markedly in their resistance to high and low temperatures. Whereas the rice weevil is predominant in the Middle and Southern States, it has not established itself in the extreme Northern States and is unable to withstand the northern winters except in protected situations. On the other hand, the granary weevil is seldom if ever found in the South below North Carolina, but is the predominant form in the Northern States. In order to obtain more precise information on the relative effects of various temperatures on the two weevils, a number of experiments were carried on in the laboratory with small refrigerator units and incubators, in which the temperatures could be controlled. The results obtained were striking and seemed worthy of record.

A constant temperature of zero Fahrenheit proved quickly fatal to both species, although the granary weevil was able to withstand even this low temperature slightly longer than the rice weevil. Adults of the rice weevil were killed after an exposure of four hours, and adults of the granary weevil after an exposure of five hours, to this temperature.

Up to a certain point, as the temperature was increased, the difference in resistance between the two species became more and more apparent. At a temperature of 5° F. an exposure of four and a half hours was sufficient to kill adults of the rice weevil, whereas an exposure of seven and a half hours at the same temperature was required to kill adults of the granary weevil.

With a temperature ranging from 15° to 20° F., adults of the rice weevil were killed in 3 days and adults of the granary weevil in 14 days. With a temperature ranging from 20° to 25° F. a majority of adults of the rice weevil were killed in 3 days, although a few showed feeble movements after an exposure of 6 days. These longer-lived individuals all died shortly thereafter, without regaining normal activity. Adults of the granary weevil were killed at this temperature only after an exposure of 33 days.

With a temperature ranging from 25° to 30° F., adults of the rice weevil were killed in 8 days and adults of the granary weevil in 46 days, or nearly six times as long. At 30° to 35° F. a majority of adults of the rice weevil were killed in 8 days; a few, however, showed faint movements after an exposure of 16 days, but died without regaining normal activity. An exposure of 73 days at this temperature was necessary to kill adults of the granary weevil.

With a temperature ranging from 35° to 40° F., adults of the rice weevil were killed in 18 days. A few specimens of the granary weevil showed faint movements after being exposed to this temperature for 111 days. With a temperature ranging from 40° to 45° F. adults of the rice weevil were killed in 80 days,

<sup>1</sup> Received for publication Apr. 19, 1924—issued Nov., 1924.

this slight increase in temperature associating itself with a marked increase in longevity. Specimens of the granary weevil were still alive after an exposure to this temperature for 105 days.

With a temperature ranging from 50° to 60° F., and under the other conditions prevailing, the normal lives of both species of weevil were greatly prolonged. Adults of the rice weevil confined in a refrigerator with this range of temperature lived for a period of 558 days, and specimens of the granary weevil in the same refrigerator for a period of 873 days. The normal life of the rice weevil in summer is from 100 to 200 days, while that of the granary weevil averages from 200 to 250 days.

The immature stages of these two weevils show a similar difference in their powers of resistance to low temperatures. Eggs of the rice weevil perished after being exposed to a constant temperature of 30° F. for 4 days, whereas eggs of the granary weevil survived an exposure to the same temperature for a period of 28 days. Larvæ of the rice weevil were killed by an exposure to a temperature of 30° F. for a period of 11 days, while larvæ of the granary weevil survived an exposure of 44 days to that temperature.

The data previously quoted have been derived from experiments with several thousand individuals. However, the susceptibility to low temperatures varies so much in individuals of the same species that very large numbers must be experimented with in order to arrive at definite conclusions regarding the lethal qualities of any one temperature. The foregoing figures can not be considered quite final until further experiments involving many more individuals have been completed. More detailed results regarding the lethal effect of various low temperatures on the different stages of these two weevils will be published later, when experiments now under way have been completed.

The effect of high temperatures on the two weevils is also of interest, although there are no very great differences in the reactions of the two species to heat. With both species constant temperatures above 95° F. soon prove fatal. Of a large number of adults of the rice weevil confined in an incubator at a temperature ranging from 95° to 98° F., all were killed at the end of 9 days. Adults of the granary weevil confined in the same incubator were all dead at the end of 13 days. A temperature of 120° F. killed adults of both species in three hours, and a temperature of 130° F. within 30 minutes. It is interesting to note that oviposition ceased at a constant temperature of 94° F.

# UNINUCLEATED AECIDIOSPORES IN CAEOMA NITENS, AND ASSOCIATED PHENOMENA <sup>1</sup>

By B. O. DODGE

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## INTRODUCTION

The cytology of the mallow rust *Puccinia malvacearum* has been studied by Werth and Ludwigs (15), Moreau (12), and others.<sup>2</sup> Nothing was discovered that suggested to these investigators that the omission of the spermogonial structures has any special significance whatever in the life of the rust. The discussions and controversies in regard to this short-cycled *Puccinia* have largely centered around questions as to time and method of infection and of over-wintering. Cell fusions take place in the sorus, each binucleated cell of the teleutospore becomes uninucleated by nuclear fusion, divisions occur and a 4-spored promycelium is developed.

In describing the spore forms of species of rusts mycologists frequently state that spermogonia (pycnia) are unknown or wanting. A preliminary study of the short-cycled orange-rust, *Caecoma nitens*, on blackberries, has led the writer to suspect that the suppression of spermogonia in the life of this rust may follow as the result of something more fundamental than that which leads to the omission of the pycnidial stage by some species of the ascomycetes. In other words, if the facts can be interpreted in the light of phylogenetic developments, it may be discovered that the omission or suppression of the spermogonial stage is in reality of sexual significance although the spermatia in themselves take no part in a process of fertilization. A brief résumé of the occurrences which led the writer to give closer attention to the cytological details in the growth of the orange-rust of *Rubus* may perhaps serve as a statement of the main features presented in this paper.

Several years ago while growing rusts in the greenhouse for class use at Columbia University, the writer observed that in certain cases of infection of blackberry by the short-cycled orange-rust, no spermogonia were developed on plants whose leaves were maturing the aecidial stage. This was in striking contrast with what is commonly known to occur. One of the most familiar sights in this country is the appearance of etiolated blackberry shoots whose leaves are covered with spermogonia when the infected sprouts emerge from the ground some days in advance of the others. In reporting on the results of work relating to infection of blackberries with the "haploid" generation of the orange-rusts (3), it was pointed out that as the inoculated plants of the Iceberg variety began to show signs of infection, no spermogonia could be found, even though the shoots were several inches high and the young aecidia were beginning to develop. That this omission of the spermogonial stage was not due specifically to peculiarities of this host, which is

<sup>1</sup> Received for publication June 30, 1924—issued Nov., 1924.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 1058.

an albino variety, was evident later when plants of the Mercereau variety similarly infected also failed to show spermogonia. Infected plants of both varieties were observed again the following year to see if spermogonia might not develop the second year after infection. At no time in the past three years has the rust on these plants produced spermogonia. This fact was the more interesting because on those plants of the Ancient Briton and Eldorado varieties which had been infected with the short-cycled rust in one of the experiments, spermogonia had always preceded the aecidia. The rust on a Taylor blackberry which was infected when received from the nursery, developed both spermogonia and aecidia, but the rust on other plants of this variety which later were artificially infected did not develop spermogonia.

During the spring of 1923, two root shoots of the Iceberg variety were inoculated with orange-rust, the one was infected by sowing sporidia from aecidiospores of the strain from the Ancient Briton which had developed spermogonia, the other Iceberg was infected with spores from rust on wild blackberry. Both plants inoculated became systemically infected and developed aecidia in 1924. The first plant showed many spermogonia on the leaves from the basal shoots as soon as they developed; the infected basal shoots from the other plant developed only aecidia, no spermogonia. While endeavoring to account for the occasional suppression of the spermogonial stage of the short-cycled orange-rust, it was discovered that aecidiospores of those strains of the rust omitting the spermogonial stage are uninucleated. Cell fusions do not regularly take place in the aecidium primordium. The uninucleated spores develop promycelia when they germinate but the promycelia are 2-celled, and each cell produces only one sporidium.

As the figures to be discussed later show, the number of cells in a promycelium is not constant for any strain. Promycelia are reported as 2-celled, or 4-celled, when one sees large numbers that are 2-celled or 4-celled, as the case may be. If no spermogonia could be distinguished with a hand lens on an infected leaf it would be reported as without spermogonia, when a more careful examination with a higher-powered microscope might prove that poorly developed or vestigial spermogonia were present. In the discussion which follows, the statement as to absence of spermogonia should be read with this understanding.

The absence of spermogonia may seem to be a point particularly stressed in the following account, when it is clear that the facts regarding the production of 2-celled promycelia which function is the feature which is being set forth as a new departure from the regular order of promycelia in the rusts. It will be shown later, however, that both of these features are merely the outward expression of something of relatively far greater significance fundamentally, and that is, that an aecidium of the orange-rust of *Rubus* may arise without cell fusions and that the spores matured without such cell fusions are generally uninucleated.

Since the long-cycled, as well as the short-cycled orange-rusts, have both been proved to be widely distributed in America on a number of species of *Rubus*, further evidence on the mere question of their distribution in the form of extensive tables showing germination tests is unnecessary, except as some new element enters the discussion. The discovery of uninucleated strains with 2-celled promycelia makes it highly desirable that further germination tests be made in such a way as to record the different types of promycelia as correlated with the relative abundance of spermogonia, as well as the size, form, and color of the spores. The abundance of the strain which develops 2-celled promycelia may be judged from the following accounts of collections gathered at random in various localities in one season.

## OCCURRENCE OF CAECOMA NITENS WITHOUT SPERMOGONIA

Miss Ruth Colvin, who was assisting the writer with this work, made one collection of orange-rust about every mile from wild blackberries May 24 along the road between Washington and Fairfax, Va. The results of her germination tests show that only 11 of the 30 collections were of the strain whose spores develop 2-celled promycelia; only one long-cycled specimen was obtained, in this case the host happened to be the black raspberry, *Rubus occidentalis*. In most regions around Washington one would certainly find on such a trip blackberries infected with the long-cycled form.

Miscellaneous gatherings on wild blackberries near Upper Marlboro, Md., always contain some specimens which are long-cycled. Twenty-two specimens were gathered from wild blackberries in this region June 2. The presence or absence of spermogonia was determined in the field by Prof. R. A. Harper and Dr. C. L. Shear. Of the 22 gatherings, seven happened to be long-cycled; the spores from the eight specimens without spermogonia produced 2-celled promycelia, and those from three gatherings developed only 4-celled promycelia. The rust on three different plants was of the type which develops spores of two sorts; some produce long germ tubes characteristic of the long-cycled forms, others germinate with 4-celled promycelia. The specimens which were proved to be of the long-cycled type were also characterized by the greater abundance of spermogonia.

The results of germination test of specimens collected May 9 at Chadbourn, N. C., and May 10 to 12 at Louisville and Vidalia, Ga., are such as to suggest that the strain which does not develop spermogonia normally and whose spores form 2-celled promycelia, greatly predominates in the South. The specimens tested, however, were gathered within a limited area in each place, so that a further survey would be necessary in order to determine this point. Of the 18 specimens tested only one was found which developed 4-celled promycelia. This was found on *Rubus trivialis* at Louisville, Ga.

Miss Colvin sent in 11 specimens gathered May 30, between Washington, D. C., and Gettysburg, Pa. No spermogonia could be found on leaves of six specimens whose spores developed 2-celled promycelia. Spermogonia were abundant on four different specimens, three of which were short-cycled and formed 4-celled promycelia. The other one was long-cycled. One specimen showed a few spermogonia and some 4-celled promycelia were found in the culture but 2-celled promycelia were much more abundant. A few other cases of infection found elsewhere have proved to be of a type where 2-celled promycelia are developed, although spermogonia are present on the leaves.

On June 3, 19 collections were obtained at the experimental farm at Bell, Md. Leaves on nine infected plants showed spermogonia and the rust was of the 4-celled promycelium type in six cases; the other three gatherings were long-cycled. Spores from nine specimens showing no spermogonia developed 2-celled promycelia. One large plant with a number of canes was clearly infected with two different strains of orange-rust. Leaves on most of the canes showed an abundance of spermogonia. Here the rust was long-cycled. No spermogonia could be found on leaves on one cane and here the rust was short-cycled, the promycelia being 2-celled (see Pl. 1, B, D).

On June 12, Professor Harper and Dr. F. D. Fromme sent in nine specimens from rusted blackberries gathered on the road to Mountain Lake, Va. No spermogonia could be found on the three specimens whose spores germinated with 2-celled promycelia. Five specimens showed an abundance of spermogonia. Only one of this number proved to be short-cycled, 4-celled promycelia being formed as the spores germinated.

Dr. N. E. Stevens, while en route by automobile from Washington, D. C., to Providence, R. I., June 4 to 6, sent in 35 specimens of orange-rust on blackberries and dewberries. The germination tests show that the strain which develops spermogonia and 4-celled promycelia was more abundant along this route at that time than was the strain without spermogonia and producing 2-celled promycelia; only 7 specimens were of the latter type, while 23 were of the type which develops 4-celled promycelia.

Whether the strain which develops 2-spored promycelia may not be relatively more abundant as one goes farther South can not be said with certainty. When the result of germination tests of rust collected from northern New Jersey and central New York are compared with those from North Carolina and Georgia, it might seem that the strain without spermogonia which develops 2-celled promycelia is more abundant in the South. All of the specimens collected at Junius, Geneva, and Albany, N. Y., June 16 to 18, showed at least some spermogonia. Only a few localities were visited but it is possible that the strain in which the spermogonial structures are poorly developed or omitted is not so common in the North. Of the 24 specimens tested 5 were long-cycled. Two-celled promycelia developed from spores from 2 specimens.

Several other collections were made at Ridgewood, N. J. Owing to the excessively hot weather prevailing and the delay in the mails, very poor germination was obtained. All of these specimens collected June 19 on wild dewberry showed spermogonia when carefully examined later. When germination was such as to enable one to determine the nature of the promycelium, it was found to be 4-celled. A few long germ tubes were seen in cultures from 6 specimens on blackberry. Very likely this rust was long-cycled and further search later will result in the discovery of telia in this vicinity.

All of the leaves in specimens collected at Wells Beach, Me., June 22, on wild dewberry and blackberry, were covered with spermogonia. Tests proved that the rust here is long-cycled. Dr. D. Folsom has sent in specimens of orange-rust collected in Maine. Germination was tested at Orono and the rust found to be short-cycled.

A number of plants of different varieties and species of *Rubus* infected with orange-rust are grown in the greenhouse at Arlington, Va., from year to year. In every case here so far tested, where spermogonia are readily seen with a hand lens, the rust is either long-cycled, or if short-cycled, it develops 4-celled promycelia.

With the results of all the germination tests reported above in mind, it is possible to make three general statements covering this phase of the question.

(1) Spermogonia are always found in considerable numbers on leaves of *Rubus* infected with the long-cycled orange-rust *Gymnoconia interstitialis*.

(2) If no spermogonia are present on a leaf showing aecidia of orange-rust the spores will be found to be uninucleated, and on germination they will form 2-celled promycelia.

(3) In strains of the short-cycled rust whose spores develop 4-celled promycelia, spermogonia always precede or accompany the formation of aecidia.

## TWO-CELLED PROMYCELIA FROM A STRAIN HAVING SPERMOGONIA

During the prosecution of this survey work there has been found very rarely a form of orange-rust in which spermogonia appear to be fairly well developed, yet the aecidiospores are uninucleated and develop 2-celled promycelia. A Crandall blackberry was found infected with this type. Some of the Eldorado blackberries in Doctor Rankin's experimental plots at Geneva, N. Y., show

spermogonia. A few 2-celled promycelia were seen in cultures from this collection. A blackberry from Doctor White's plot at the Arlington Experiment Farm in Virginia regularly shows rust with spermogonia, yet practically every spore produces a 2-celled promycelium. Sections show that the spores are mostly uninucleated. One other specimen sent in appears to be of the same nature. So far as has been learned, every specimen of this interesting form occurred on a cultivated variety of blackberry. It is the only form with spermogonia so far studied that has uninucleated aecidiospores.

#### CHARACTERISTIC FEATURES OF PROMYCELIA OF THE SHORT-CYCLED ORANGE-RUST

In the preceding discussion, in order to avoid confusion, no mention has been made of the great variability which may sometimes occur in the form and size of the promycelia which develop when spores from a single aecidium are germinated. Likewise, the promycelia were said to be 2-celled or 4-celled, as the case might be, disregarding the fact that in certain germination tests promycelia with from 3 to 8 cells were found. Furthermore, the number of cells which a promycelium develops does not necessarily correspond to the number of nuclei visible or to the number of sporidia that finally mature. Various types of promycelia, as they appeared in the germination test cultures, will be described. The method of origin of the chains of aecidiospores and the behavior of the nuclei during germination and formation of sporidia will then be considered.

As a rule, the aecidiospores of the strain which seems to omit the spermogonial stage are smaller than those of the long-cycled form. The color characters of the aecidia are not always dependable, but, in general, when the aecidia are rather light yellowish-orange as contrasted with reddish-orange colored (Pl. 1, B, D) the spores will be found smaller, and less uniform in shape and size. These small spores are the ones that regularly produce 2-celled promycelia on germination. The more common types of this promycelium are shown in Plate 5, A to I.<sup>3</sup> Two septa are usually plainly visible, cutting off two cells, leaving the spore and a short piece of the germ-tube without cytoplasmic contents. The promycelia are rather slender and their sporidia are not large. As compared with these structures developing from the large spores matured by other short-cycled strains, they are certainly smaller. In cultures of germinating spores from plant No. 497 no promycelia were found with four sporidia attached. Plate 5, H, shows three sporidia on a 2-celled promycelium which is rather "abnormal". Sometimes by following this type of growth a little further, one will find that the contents of one of the sporidia will disappear so that only two finally are discharged. The sterigmata are frequently long and filamentous (Pl. 5, D). This seems to be due to the conditions under which the spores germinate. Normal sporidia are finally developed.

The more common types (4-celled) of promycelia developed from spores of strains producing spermogonia are shown in Plate 5, J to M. These are 4-celled and will all develop four sporidia.

Certain odd types are also figured. They are, however, perfectly normal. A 9-celled promycelium is shown in Plate 5, N. Some of the cells are dead or empty but six sporidia are being matured. Forked promycelia are not very rare. Figure R shows one with eight cells, six of them alive; one sporidium has been discharged, two more are matured, and one is just forming.

The influence that the conditions attendant on spore germination have on the form of the promycelia is occasionally very astonishing. For example, the spores from plant No. 569 develop in the warm laboratory very long germ

<sup>3</sup> The 4-mm. dry lens was used in drawing the figures in Plate 5; the 1.5-mm. oil immersion lens for Plates 2 to 4, inclusive.

tubes (Pl. 5, V), resembling those of the long-cycled *Gymnoconia*. They become 4-celled, however, and mature four sporidia. Plate 5, S, shows such a promycelium as it would appear a little later. Five-celled promycelia (Pl. 5, T) with one sporidium from each, are sometimes seen in cultures. The formation of five cells and five nuclei after a reduction division is not the regular order of procedure as commonly understood. These odd types of germination are referred to in order to focus attention on the question of nuclear behavior during and after aecidiospore formation, and to raise the question as to whether or not nuclear fusions followed by reduction divisions are always to be found in the short-cycled orange-rusts.

While every specimen of the rust so far discovered which omits its spermogonial stage is characterized by 2-celled promycelia, as previously noted, there are certainly clear-cut cases where spores from leaves with spermogonia are uninucleated and develop 2-celled promycelia with only two sporidia (Pl. 5, W).

#### ORIGIN OF AECIDIA WITH BINUCLEATED AECIDIOSPORES

In the light of what is known of the vagaries of *Rubus* orange-rusts, it is impossible to say what was the nature of the material which Olive (13) used as the basis for his cytological work on the orange-rust. It was gathered in Indiana on *Rubus* sp. He figures the Christman cell fusions referred to as "sexual fusions." The rust that Olive studied evidently conformed in every way to what might be expected of the long-cycled form *Gymnoconia*.

Kunkel (5) presumably did not find anything in his studies of the development of the aecidial sorus of the short-cycled form of the orange-rust to suggest that there might be at times some departure from the regular order of procedure in the growth of the aecidium. According to him the two nuclei originally found in the aecidiospore fuse before germination of the spore and as the germ tube begins to push out the fusion nucleus divides, sometimes while yet in the spore; the first division may also occur in the promycelium. After the second division the cross walls are laid down so that four uninucleated cells comprise the mature promycelium. Each of the four cells then develops a sporidium containing a single nucleus. In the manner of their origin, in the behavior of their nuclei on germination, and in the type of promycelia formed from them, the aecidiospores of *Caecoma nitens* were thus, according to Kunkel, not unlike those of *Endophyllum sempervivi* as reported by Hoffman (4) and others.

The writer's studies have been confined to preparations made from material obtained from plants infected with rust whose spores have been germinated.

There are many interesting features in the development of aecidia which need further attention other than the so-called "sexual fusions," but as the question relating to the ultimate form of the promycelium is in the main determined at the origin of the aecidiospore, it will be necessary to give this phase of the subject some attention. Disregarding what occurs when the aecidia of the long-cycled orange-rust are formed (Pl. 2, T), the origin of the chains of spores in those strains where 4-celled promycelia are regularly developed from aecidiospores will first be referred to. Cell fusions (Pl. 2, M to S) in numbers can be found in sections of aecidial primordia of the rust on an Iceberg blackberry No. 543. Spermogonia were developed in large numbers on this plant and germination tests showed that most of the spores germinated as short-cycled, forming quite regularly 4-celled promycelia. Binucleated spores and intercalary cells are cut off in regular order above the fusion cells.

In no case is there any indication in these sections of a fusion of the two nuclei in a spore. Hoffman (4) shows that in *Endophyllum sempervivi* the two nuclei



fuse and then often undergo the first division before the spore is shed. The writer's sections of telia of the long-cycled form *Gymnoconia* show that the two nuclei fuse as the teleutospore reaches maturity. Is it possible that the two nuclei of the short-cycled orange-rust spore never fuse? If so, the reduction division would be omitted, only that standing in place of the homoeotypic division persisting to give the four nuclei of the "normal" promycelium.

In some preparations from an infected loganberry "two-legged" cells are very difficult to find (Pl. 2, L). Very likely such figures represent sections at right angles to the pairing cells so that only one of the "leg" cells appears in the section. It is not impossible that chains of binucleated spores may sometimes, or even regularly, arise by mere nuclear division as claimed by Kursanov (8). It is certainly strange that fusion cells are so hard to find in this strain on loganberry.

Rust from a blackberry also shows aecidial chains of 2-nucleated spores (Pl. 2, I, J) where it is also difficult to understand why more examples of two-legged fusion cells are not to be found. The cytology of this form of the orange-rust is being given further attention.

#### ORIGIN OF SORI WITH CHAINS OF UNINUCLEATED AECIDIOSPORES

There being no question as to the existence of cell fusions, at least in the aecidium primordium of the *Gymnoconia* orange-rust, the form of rust on blackberry which omits the spermogonial stage will next be considered.

Plate 2, A, represents merely in outline and position the structures in an aecidial sorus of the rust on Crandall blackberry which was obtained May 17, 1922. The uninucleated basal cells arise from uninucleated cells of the sorus primordium. Intercalary cells are cut off and the spores are matured in a normal fashion. There were no cell fusions in this sorus. At B a chain of 2-nucleated spores and interstitial cells is shown adjacent to chains of uninucleated spores. Kursanov (6) says, in effect, that in *Aecidium leucospermum* this would mean that the nucleus of the original basal cell simply divided and thereafter 2-nucleated cells are cut off. In Plate 2, D and F, are shown chains of spores in an aecidium from plant No. 497, most of whose spores produce 2-celled promycelia. At F the two lower cells in the chain are clearly uninucleated while the spores above are binucleated, but at E the basal cell is binucleated. In some forms scarcely a binucleated spore can be found in a sorus. At G is shown a very young sorus, and at H, a somewhat older one where from first to last the cells are uninucleated.

As noted, cell fusions regularly occur in the aecidium of the long-cycled orange-rust (Pl. 2, T). By triple or quadruple fusions, 3 and 4 nucleated spores presumably may arise as in the other rusts. What would this mean in germination and in infection? In forms of the short-cycled *Caeoma* rust with spermogonia where cell fusion would be expected to occur frequently, binucleated spores may possibly arise without such fusions. Then, too, 3-nucleated spores are not at all rare in these forms (Pl. 2, K). In strains without spermogonia and with uninucleated spores originating without fusion there are also 2-nucleated spores, possibly formed as the result of nuclear division and also from cell fusions. By studying stained preparations of germinating spores the writer becomes more thoroughly convinced that the orange-rusts represent forms which are not fixed in their life cycles. Bearing in mind the facts regarding the methods of origin of the aecidiospores one may understand more readily why there is such a great variation in the forms of promycelia.

## NUCLEI IN PROMYCELIA

In Plates 3 and 4 are shown promycelia in various stages of growth from the germination of spores on to the formation of sporidia. The preparations studied were fixed and stained. The shaded areas merely represent living substance, cytoplasm and nuclei. The magnification is the same as used in Plate 2, which is somewhat higher than used in Plate 5, with which the figures should be compared. In Plate 3, A to K, are represented promycelia from spores of the rust on plant No. 497. At A and B division of the original nucleus has taken place; at C, the original nucleus has moved out into the promycelium where it will divide only once. The first cross wall is sometimes laid down before the first division (D), but most frequently division occurs in the spore itself. Any departure from standardized behavior on the part of the nuclei is reflected at once in the promycelium (J, K). In one case the promycelium has only two cells, but one of them has two nuclei. In the one shown at K there are three cells, with one, two, and three nuclei, respectively.

The other promycelia (Pl. 3, L to R), are from spores in aecidia of strains which produce spermogonia. It should be noted that spores which develop promycelia with three or more cells are correspondingly larger. At M is shown a promycelium with six cells, but only four of them are alive and nucleated, while at Q there is shown a 4-celled promycelium, one cell of which is dead, the other three contain two nuclei each. At N is shown a sporidium with three nuclei; one cell of the promycelium was dead. Perhaps its nucleus migrated into the upper cell and entered its sporidium. A binucleated sporidium arises as the result of division of its original nucleus, generally after the sporidium is matured.

Further details in the growth of promycelia are shown in Plate 4, A to J, from plant No. 489, where most of the aecidiospores are uninucleated. At A, the germ tube is put out before nuclear division, while nuclear division has already occurred in the spore shown at B. At C, the two daughter nuclei are passing out of the spore. Nuclear division is shown taking place in the promycelium at D. In cultures of this rust from plant No. 489 a great majority of the spores germinate in the forms shown from A to K. Figures L to S show types of germination from spores which had originally two or more nuclei. Three nuclei were clearly visible in the large spore shown at L. One nucleus in each of the cells of promycelia shown at M, Q, and O is degenerating. At N, two nuclei are entering the sterigma. The central cell of the 3-celled promycelium at S has two nuclei, the others, one each. Figures T to V in Plate 4 show the prevalent type of promycelium from the rust on plant No. 493. At X and Y are shown 4-celled promycelia, but it seems two cells in each case have captured the nuclei, three and three at X, two and four at Y. If the terms "normal" and "abnormal" must be used in describing promycelia, it should be done for convenience only, meaning by "normal" the most common or prevailing type. All of the odd forms could probably be looked upon as regular if one only knew the whole story. The writer has found aecidiospores with as many as seven nuclei in a young sorus whose spores were most regularly uninucleated.

## DISCUSSION

The short-cycled orange-rusts of *Rubus* have much in common with those included under the genus *Endophyllum*. According to Hoffman (4), whose work has been confirmed by Moreau (12), cell fusions occur at the origin of the aecidiospore chains in *E. sempervivi*. The two nuclei resulting from the conjugate divisions of the nuclei contributed by the fusing cells, being cut off in the spore cell, fuse before the spore is shed. The fusion nucleus, which is much larger than either of the original nuclei, then undergoes a division so that the mature spore

again contains two. This first division may be delayed and later occur in the germ tube. The first septum is laid down before the second division occurs. Finally, a single uninucleated sporidium develops from each of the four cells. Such a course of procedure, following as it does what is known of nuclear behavior during the formation and germination of the teleutospore of the rusts, has been considered by some authorities to be sufficient evidence to warrant calling the aecidiospore of an *Endophyllum* a teleutospore. Evidence is accumulating very rapidly, however, to indicate that while the spore forms of the rusts as morphological entities are fixed because of their phylogenetic antecedents, the methods by which a particular spore form arises and the nuclear behavior at its origin and during germination are far from generalized, orderly, or fixed processes.

In the Florideae the methods of origin of the cystocarps and nuclear behavior in the cell activities involved in the formation of carpospores vary in some essential details with almost every genus in the five orders. One certainly must expect that their rust offspring will be just as individualistic.

There is an *Endophyllum*, *E. euphorbiae-silvaticae*, in which, as proved by Sappin-Trouffy (14) and Moreau (12), the two nuclei originally entering the aecidiospore never fuse. They pass out into the young promycelium and divide once, giving the four nuclei for the maturing of four sporidia. According to Maire (10), one of the two original nuclei of the spore of *E. valerianae-tuberosae* degenerates so that a reduction division could not follow.<sup>4</sup> Moreau (11, 12) has furthermore given convincing proof that there is a form of *E. euphorbiae* with uninucleated aecidiospores and that there are no cell fusions in its aecidium. Her figures of the germinating spores and of the promycelia are, however, not all that could be desired. Whether or not Moreau believes that the promycelia from these uninucleated spores would "normally" be 4-celled and produce four spores is not clear from her papers. Nothing in her figures suggests such a thing.

Certainly one finds no explanation of the peculiarities of the orange-rust in *Puccinia malvacearum*, the only rust lacking spermogonia that has been thoroughly studied as to its cytology and physiology. It is a perfectly "normal" short-cycled form, except for its lack of spermogonia. In the absence of any definite knowledge respecting spore formation and the nuclear phenomena in those other rusts which lack spermogonia it would be futile to speculate on the meaning of the loss of these structures in the strain of *Caeoma nitens* under discussion.

Knowing nothing as to the development of spermogonia by the "*uninucleatum*" strain of *Endophyllum*, and nothing regarding the number of nuclei and cells in the promycelium except that the germ tubes that were obtained did have septa, it can only be said that further research may show that this *Endophyllum* may resemble the orange-rust not only as to the origin of its uninucleated spores, but also in the suppression of its spermogonia as well as in the formation of 2-celled promycelia. Germination of the spores of the form of *E. euphorbiae*, which Maire (10) claims becomes uninucleated by degeneration, should also be studied.

Two other European cytologists are accumulating evidence proving that life histories of species of rusts are not always as fixed events as has heretofore been generally believed. Kursanov (?) has pointed out that in certain sori of *Aecidium punctatum* the peridial cells and their interstitial cells are uninucleated. The spores in such a sorus are uninucleated except that here and there a spore has two nuclei clearly as the result of nuclear division. Nothing is said of the manner of germination of the uninucleated spores. There is a form of *Aecidium*

<sup>4</sup> In a later paper (MAIRE, R. L'ÉVOLUTION NUCLÉAIRE CHEZ LES ENDOPHYLLUM. Jour. Bot. [Paris] 14:80-97, 369-382, pl. 3. 1900) he figures 2-celled promycelia, failing to furnish any evidence by figures, that the aecidiospore becomes uninucleated by degeneration.

*leucospermum* which occurs frequently in the vicinity of Moscow and which Kursanov (6) finds has sori with chains of uninucleated aecidiospores. No cell fusions take place at their origin. The spores germinate like the ordinary aecidiospore, except that the germ tube has one nucleus instead of two. The spores with two nuclei are much larger than those with only one, whereas Moreau (12, p. 182) found that the uninucleated spores of her *Endophyllum* corresponded in size with those described for the species. Occasionally a spore in one of the chains is binucleated. Such a case is clearly the result of a nuclear division. Where a chain of binucleated spores is found in a sorus the possibility of a cell fusion at the base is not precluded.

Lindfors (9) has more recently given a most interesting series of brief discussions of life histories of certain rusts. He has signally departed occasionally from the practice of trying to make what he finds in his material conform to the commonly accepted notions of cell fusions and to the doctrines relating to the fixity in rust cycles. For example, he finds that in *Uromyces acetosae* the first telia develop close to the aecidia. He figures an aecidium and a telium arising out of a common uninucleated mycelium. He also claims that the binucleated condition is not limited as to its origin, to cell fusions at the base of a sorus. Although cell fusions occur in *Puccinia arenariae*, without spermatogonia, the promycelium has only two cells. Two binucleated sporidia are formed.

Up to the present time one of the greatest obstacles to the understanding of the workings of evolution in the Uredineae is our proneness to believe in the fixity of what are called genera and species. Owing to the immediate need for some systematic treatment of rust parasites, which were being discovered attacking our economic plants, much of the preliminary work, of necessity, has been based on dried material. Furthermore the cytological work has mostly been confined to a study of the so-called sexual fusions. The desirability of gathering, for fixation, material obtained as the result of extended culture work was clearly not of prime necessity in those days when Blackman and Christman were making their discoveries relating to nuclear migrations and cell fusions occurring at the origin of the aecidium. Moreau and Kursanov did not supplement their cytological work on uninucleated aecidiospores with culture work, and nothing was said regarding the presence or absence of spermatogonia in those forms. Judging from Moreau's figures of the promycelia, which do not show any nuclei, it would not be surprising to find that if the uninucleated spores of that *Endophyllum* were germinated under conditions sufficiently normal to lead to the formation of sporidia, the promycelium would be found to be regularly 2-celled with one nucleus in each cell.

Blackman (1) saw in the sterile cell above one of the fusing cells the ancestral red alga trichogyne, and he saw in the migrating nucleus passing up into the basal cell (the "egg cell") a substitute for the now functionless spermatium. This view has long been discarded. In all of the more or less deep-seated aecidial primordia the fusing cells are in reality merely intercalary cells of the stroma-like, space-making, and food-storing hyphal plexus. The fusions are of exactly the same nature as are those occurring between the accessory sterile and auxiliary cells in the red algae. The resultant is the accumulation of materials and vigor for the building of numbers of reproductive spore bodies.

Christman's (2) suggestion that the cell fusions occurring between two equal cells must represent the sexual fusions of the ancestral *Spirogyra-Mucor* of the Conjugatae, while not generally accepted in this particular sense, nevertheless has gone far to establish firmly in the literature the terms sexual cells (gametes) and sexual fusions for those purely secondary fusions occurring between cells which have no homologies whatever to sexual organs, spermatia and oogonia, of any possible ancestral form.

There is no structure known in the rusts which takes the place of or is the homologue of an egg apparatus. The factor which stands for such an organ was either discarded when the rusts were evolved, or it became recessive to the extent that, for all that is known, the egg structure is always suppressed. It is this loss or impotency of the factor for that element of femaleness represented by the egg that distinguishes a rust most fundamentally from its ancestral alga.

The existence of a strain of any rust characterized by the development of a 2-celled-promycelium has not hitherto been reported. Inasmuch as the suppression of spermogonia has been said in this paper to be so constantly associated with the production of uninucleated spores with abbreviated promycelia, it might appear as though the writer looks upon the production of spermogonia as inseparably connected with the faculty for initiating cell fusions. This may indeed be true to a certain extent, but, as has been noted, the development of spermogonia does not always insure cell fusions, for a few cases of infection have been found where, though spermogonia were present, the spores were uninucleated.

There is, of course, something in the inheritance of the rusts that determines when and where these cell fusions shall take place, as well as the nature of the cells fusing. The five great orders in the Florideae are distinguished mainly on the basis of the form and disposition of the carpogonial branches and auxiliary cells, and on the nature of the cell fusions which follow as secondary events. The gonimoblasts or ooblastema filaments, sporophytic outgrowths from the fertilized egg, in many genera are also involved in these secondary fusions preliminary to the development of carpospores. It is to these secondary cell fusions in which the auxiliary cells of the red alga take the leading part that we must look for the homologues of the rust fusing cells.

There being no organ such as an egg apparatus in the rusts, the spermogonia, though they are so well developed in many species, can not carry out their primary male sexual function. Nevertheless, there may be a series of activities in the growth of the rust bound up ("linked") with or influenced by the very inheritance which manifests itself morphologically in the shape of spermogonia. The fusions between the ooblastema filaments and auxiliary cells, and the other cell fusions in the red algae may very well be determined by the stimulus resulting from the presence of some of that element of maleness normally derived from the spermatium during fertilization. In the absence of the sexual fusions certain auxiliary cell fusions would not occur.

Femaleness in the red algae outwardly expresses itself primarily in the organization of the carpogonial branch bearing the egg cell and its trichogyne. Auxiliary cells are a secondary manifestation or accompanying phenomenon. In a dioecious alga one should not expect to find auxiliary cells borne on the male plant. Femaleness in the orange-rust—that is, the power to develop an egg apparatus—has been lost. Auxiliary cells represented by the cells in the sorus primordium are the secondary expressions. These cells are capable of fusing, usually in pairs, under a certain stimulus.

Maleness in the red algae is expressed in the form of antheridia (spermogonia) producing spermatia. These bodies function primarily in fecundation, and, in certain groups, secondarily in the cell fusions subsequent to fertilization. In the rusts maleness has persisted as shown by the development of spermogonia with their spermatia, which are primarily functionless in the absence of an egg apparatus. This condition is associated with the occurrence of accessory cell fusions, culminating in *Gymnoconia* in nuclear fusions in the teleutospore.

A long-cycled rust becomes short-cycled without losing the power to develop that morphological structure, the promycelium. A promycelium may be formed without there having taken place a nuclear fusion previously, in which case

obviating the necessity for a reduction nuclear division. For this reason the number of cells in a rust protobasidium need not always be four. The presence of some male element exercising its secondary function indicates maturity and cell fusions will occur, while inhibition of such a development is manifested by the omission of cell fusions.

Aside from the immediate developments dependent directly on fecundation, there is frequently a whole series of activities well known and recognizable in sexual reproduction in plants and animals which result from the expression of sexuality in a secondary capacity. One can conceive of such a thing as the agent inaugurating cell fusions in the short-cycled orange-rust being ordinarily "linked" with or dependent on the factor which stands for spermatogonial development, yet occasionally becoming separated from this factor. Ordinarily then the appearance of spermatogonia would be followed by cell fusions, except where there has been a transfer of the factors for fusions to some other determinant where it could no longer express itself normally.

The Gymnoconia as noted becomes short-cycled by omitting or suppressing the telial stage. If that combination of factors which was instrumental in bringing about a shortening of the life cycle were unconnected with maleness in any way, the rust would develop its spermatogonia, cell and nuclear fusions would follow as before, and the spores would germinate with 4-celled promycelia. If one should be able to prove that in the short-cycled form there are no nuclear fusions and that chains of binucleated or multinucleated spores may arise without cell fusions, it would be further evidence of the complexity of the factors involved in the development of the various spore forms.

The writer has pointed out that the spores with one nucleus are smaller than than those with two or more. This agrees with what Kursanov (8) says of the uninucleated spores of *Aecidium leucospermum*. Kunkel (5) figures uninucleate aecidiospores of the short-cycled orange-rust, but he interprets them as resulting from nuclear fusions. It should be noted that in his figures the uninucleated spores are in both cases much smaller than the binucleated spores.

The very fact that promycelia with irregular or odd numbers of cells—three to nine are found—and that as many as five nuclei may occur in one cell, would indicate that this multicellular, multinuclear condition has not arisen as a result of fusions of nuclei in pairs in the aecidiospore followed by regular reduction divisions.

It is inconceivable that a 7-nucleated aecidiospore which was found in an aecidium, the other spores of which were mostly uninucleated, could have been the result of the fusion of seven cells. When such a spore germinates, a promycelium is formed which, from the nature of its origin, will be normal; but from a viewpoint fixed by previous misconceptions, will be abnormal. The writer has found that in certain strains of the short-cycled orange-rust practically every promycelium is 4-celled. This can almost be determined in advance by the form and size of the aecidiospores. In any culture where the spores vary considerably in shape and size some "abnormal" promycelia will be developed.

There is a rather fascinating hypothesis which might be brought forward to account for the frequency with which one finds the omission of the spermatogonial stage is associated with the omission of cell fusions and with the production of 1-nucleated spores in the aecidium.

If as Kniep maintains in the anther smut, *Ustilago antherarum*, the two sporidia at one end of the promycelium are "plus" and the other two "minus," then the host can be infected so that the rust develops normally only when a sporidium of each sort takes part in the infection. Infection from a single sporidium might be possible only in case the strain happened to be capable of, say, an androgenetic development assuming that + and - refer to femaleness and maleness. In this

event, spermogonia would be formed, but cell fusions being omitted the aecidiospores would be uninucleated. If the sporidium from the opposite end produced infection it could only be by a gynogenetic process. In such an event, no spermogonia would appear, no cell fusions would occur, and 1-nucleated spores would arise.

The two strains evolved should remain or continue to be true to form, but by bringing about a double infection with a sporidium of each sort one would obtain a strain with spermogonia, cell fusions, 2-nucleated aecidiospores and 4-celled promycelia. The difficulty with which one is able to infect a blackberry systemically and the rareness with which natural systemic infections occur in comparison with the vast number of spores shed, would suggest that this hypothesis should not be entirely ignored. One is reminded that future infection work with the orange-rusts should be carried on with spores at least from a single plant and ultimately with single aecidiospores and even with single sporidia.

### SUMMARY

Certain strains of the short-cycled orange-rust on *Rubus* do not develop spermogonia except perhaps as they are aborted or vestigial.

The aecidiospores in such strains are for the most part uninucleated. Binucleated or multinucleated spores are sometimes present in such aecidia.

Cell fusions do not occur at the origin of a chain of uninucleated aecidiospores.

The number of nuclei in an aecidiospore may be increased by nuclear divisions.

The size of an aecidiospore varies with the number of nuclei present at its maturity. The uninucleated spores are much smaller than those with two or more nuclei.

A spore with one nucleus germinates normally; its nucleus divides once so that a 2-celled promycelium which matures two sporidia is formed.

The number of cells in a promycelium and the number of sporidia matured is not fixed, but depends to a great extent on the number of nuclei in the aecidiospore. A polynucleated spore is very large and would probably give rise to a promycelium with more than four cells and very likely some of the cells would contain more than a single nucleus.

Degeneration of nuclei sometimes occurs in the cells of promycelia and certain of its cells may die, so that the number of sporidia eventually formed does not necessarily correspond to the number of nuclei originally present.

So far as yet observed spermogonia are always matured by the long-cycled rust *Gymnoconia interstitialis* and cell fusions occur.

Strains of the rust which commonly form 4-celled promycelia when the aecidiospores are germinated always develop spermogonia. In this form the binucleated aecidiospores arise as the result of cell fusions.

That nuclear fusions always occur in the aecidiospore or its promycelium in the short-cycled orange-rust is questioned.

So-called "abnormal" promycelia with other than the traditional number, four, cells are accounted for by nuclear behavior during spore development and germination. When a spore has but one nucleus, that nucleus divides only once during the formation of the 2-celled promycelium.

Binucleated spores arising from cell fusions usually develop 4-celled promycelia, possibly without nuclear fusions followed by reduction divisions. On this principle a precocious division of the nuclei in a spore followed by a multicellular promycelium need not introduce a new element into the inheritance of the rust.



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PLATE 1

A to E.—Types of orange-rust on *Rubus*

A.—Short-cycled, spermogonia and four-celled promycelia. Waxy spores heaped up in the sori.

B.—Short-cycled, no spermogonia, 2-celled promycelia. Yellowish spores waxy.

C.—Three yellowish short-cycled sori like those in B, other sori long-cycled.

D.—All sori long-cycled.

E.—Light orange empty sori, long-cycled; reddish orange sori with waxy spores are short-cycled. Two long sori partly short-cycled and partly long-cycled.  
x 12.

B. C. and D from the same plant.



A



B



C



D



E

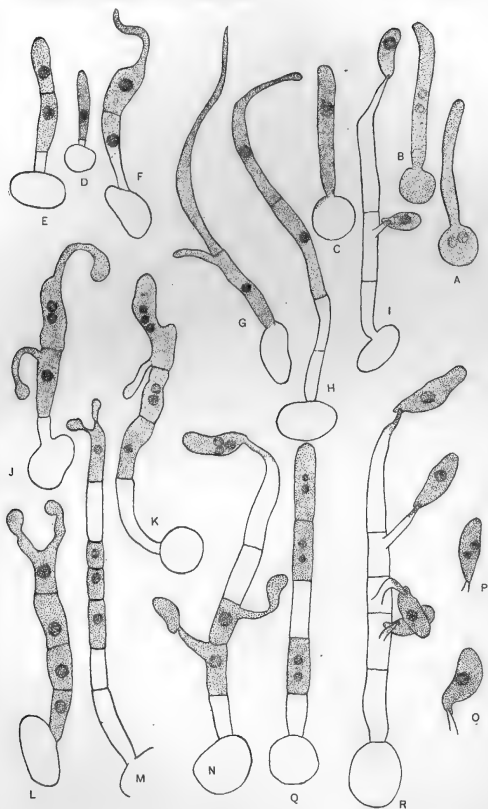


## PLATE 2

A to C.—From sections of a young *aecidium* on Crandall blackberry. No Spermogonia, no cell fusions, mostly 1-nucleated spores. D to F.—Spore-chains from *aecidium* on plant No. 497. G.—A young primordium; H.—Older sorus; all cells 1-nucleated. I to S.—From strains maturing spermogonia. T.—Spore-chain from the long-cycled *Gymnoconia* on *Rubus occidentalis*

**PLATE 3**

**A to J.—From the short-cycled rust on plant No. 497 (cf. Pl. 5, A to I). K to  
R.—From strains producing spermogonia**



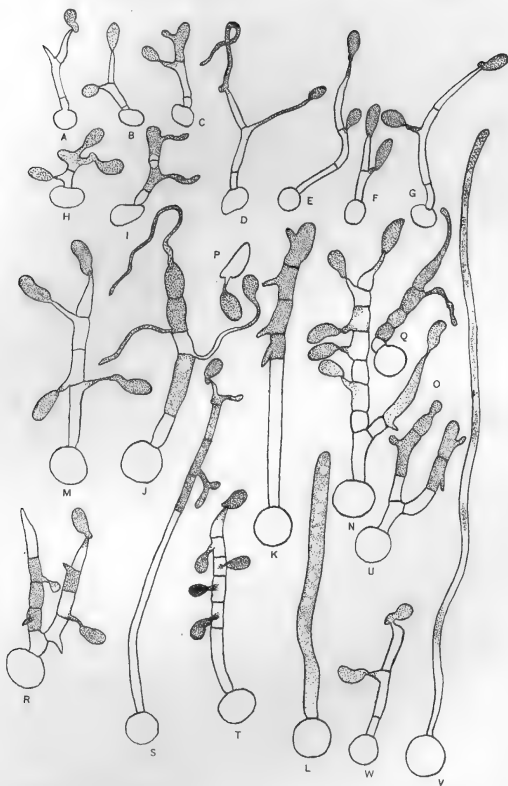


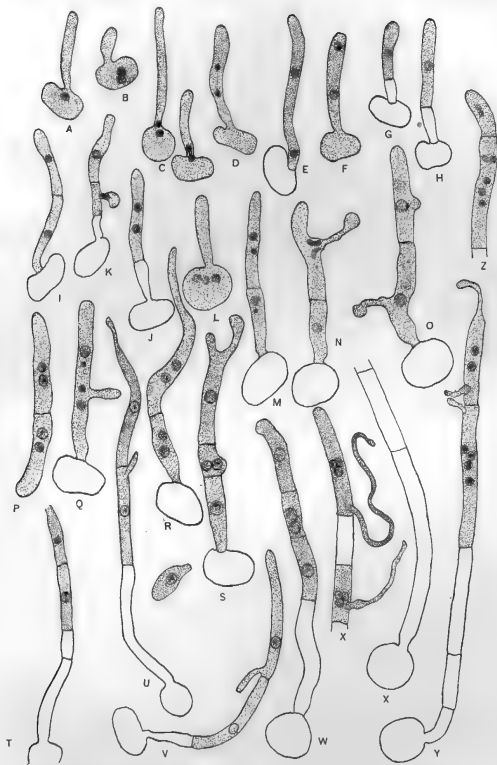
PLATE 4

A to Q.—From short-cycled rust on plant No. 489. R to Z.—From a strain of the short-cycled rust on plant No. 493.



# PLATE 5

A to I.—2-celled promycelia from germination tests from plant No. 497. Small spores slender promycelia. J to V.—Promycelia from strains which develop spermogonia. Cells without cytoplasmic contents not shaded. W.—2-celled promycelium from a strain having spermogonia and 1-nucleated spored.



# JOURNAL OF AGRICULTURAL RESEARCH

## CONTENTS

On Some Plant Parasitic Nemas and Related Forms G. STEINER	Page 1089
The Influence of Environment on Sex in Hemp, <i>Cannabis sativa</i> L. HUGH C. McPHER	1097
Spacing Experiments with Acala Cotton in Southern California H. C. McKEEVER	1081
Correlation Among Quantitative Characters in Maize J. H. KEMPTON	1095
Effect of Fumigation upon Heating of Grain Caused by Insects E. A. BACK and E. T. COTTON	1103
The Nitrification of Phosphorus Nitride F. E. ALLISON	1117
Aeial Stages of the Leaf Rusts of Rye, <i>Puccinia dispersa</i> Erikss. and Henn., and of Barley, <i>P. anomala</i> Rostr., in the United States E. B. MAINS and H. S. JACKSON	1119
Fall Sowing and Delayed Germination of Western White Pine Seed W. G. WAHLENBERG	1127
The European Hare ( <i>Lepus europaeus</i> Pallas) in North America JAMES SILVER	1133
Growth on Cut-Over and Virgin Western Yellow Pine Lands in Central Idaho C. F. KORSTIAN	1139
Some Factors Affecting Reproduction after Logging in Northern Idaho J. A. LARSEN	1149
The Effect of Cyanamid and Related Compounds on the Number of Microorganisms in Soil F. E. ALLISON	1159

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## ON SOME PLANT PARASITIC NEMAS AND RELATED FORMS<sup>1</sup>

By G. STEINER

*Nematologist, Bureau of Plant Industry, United States Department of Agriculture*

### A CEPHALOBUS LIVING IN THE GREEN LEAVES OF PHLOX DRUMMONDII

#### PREVIOUS FINDINGS

Few *Cephalobi* have been mentioned as infesting plants. De Man (see Ormerod 13),<sup>2</sup> found oats appearing as if infested by *Tylenchus dipsaci* Kühn, to harbor instead many *Cephalobus rigidus* (A. Schneider). Ritzema-Bos (15 p. 327) observed in *Allium proliferum* infested with *Tylenchus dipsaci* and other nemas, specimens of *Cephalobus rigidus* and *elongatus* de Man. In 1891 (16) he again found two *Cephalobus* species in the healthy, as well as in the diseased, parts of strawberry plants suffering from cauliflower disease, caused by *Aphelenchus ormerodis*, and considered one species closely related to, or identical with, *C. nanus* de Man, and the other with *C. rigidus*. In some plants the latter was more numerous than the *Aphelenchus*; but since all contained *Aphelenchus*, and only a majority *Cephalobus*, the origin of the disease was attributed to the former. In the first important study of a plant-attacking *Cephalobus*, 1906 and 1909, Kati Marcinowski (8, 10) showed by many experiments that *C. elongatus* enters healthy plants (rye, etc.) and, if numerous, may kill, or at least injure, the host plant.

#### NEW OBSERVATIONS

August, 1923, Florence Hedges submitted mature Phlox plants from Quaker Neck, Chestertown, Md. The plants were green with only a few brown spots, but dwarfed, less bushy, and with fewer flowers than in previous years. Larval and adult specimens of *Cephalobus* were fairly numerous within the green leaves and stems of these plants, but not massed. When the leaves were covered with water, many nemas soon left them and sank by hundreds to the bottom.

The *Cephalobus* proved to be *C. subelongatus* Cobb (3), a species based on a single female. Dr. N. A. Cobb placed at the writer's disposal a sketch of a male tail end, and notes in which this species is mentioned as living on green pepper pods, Philippine Islands, on clover seeds, Idaho, and on lily of the valley roots (*Convallaria majalis*), Washington, D. C. He has also observed *C. subelongatus* on diseased germinating rubber seed (*Castilloa*) in Hawaii, and in crowns of diseased alfalfa plants from New Jersey and Pennsylvania.

<sup>1</sup> Received for publication Jan. 28, 1924—issued November, 1924.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," pp. 1065-1066.

## TECHNICAL DESCRIPTION

Supplementing Cobb's description: The two lateral wings may be rather well developed or more or less lacking. Each of the three main lips (one dorsal and two ventro-submedial) separated from each other by a rather deep incision (Pl. 1 B), is again divided by a shallow concavity into two points, each with a papilla (Pl. 1, A and C). The two lateral points of the ventro-submedial lips, somewhat smaller than the others, apparently bear the amphids. They are very difficult to see, open behind the lateral lips, and are slightly shifted dorsad (Pl. 1, Aa and C and Pl. 4, E and F). The exceedingly narrow opening is circular or oval-shaped, and leads into a long, somewhat conical cavity (amphidial pouch). Within the latter a small number of fine fibers, possibly six, can be seen; they show spindle-shaped swellings (Pl. 4, Ca and D) and are very similar to the elements in the chemical sense organs of other animals (organs of taste as well as organs of smell). It may, therefore, be quite safe to regard these amphids as the chemical sense organs of the present species until a better or more exact explanation can be given. The figures (Pl. 4, C, D, E, and F) make any longer description needless. A front view shows six papillae; the lateral are slightly smaller (Pl. 1, E). The mouth cavity (Pl. 1, A and E) agrees with Cobb's data, but it differs from sketches of *Cephalobus elongatus* by De Man and Marcinowski.

The cardiac valvular apparatus (Pl. 1, D) consists of three groups of cutinized parts; the valves have the usual ribbed structure (Pl. 1, D); two other groups of three elements each occur farther back (Pl. 1, D, valves 2 and 3). These drawings are from live specimens. Any given circumference of the intestine comprises only two cells (Pl. 1, F).

Examination of over 30 specimens showed the excretory pore usually ventrad of the middle of the cardiac bulb, seldom fore or aft; the alimentary tube being capable of forward and backward movement, the relative position of pore and bulb changes somewhat even in the same specimen. The well developed outlet leading to the excretory pore is connected with at least one large and two or three smaller cells ventrad of the beginning of the oesophagus (renette cells or ventral glands).

Cobb described the female sexual apparatus. Plate 2, A, shows the part of the uterus which serves as the *receptaculum seminis* with the sperms in an interesting arrangement. Each relatively large sperm consists of a transparent part, well separated from a straight-edged, granulated part, and along this edge the rod-like granules are all similarly placed. In many other nemas the spermatozoa take on a definite orientation within the receptaculum, as if influenced by some tropism; the head is then directed towards the outlet of the oviduct and the sperms are massed together as if competing for the place nearest the arriving egg. Here no such condition of the sperms can be seen; as Plate 2, A shows, the clear transparent part of the spermatozoa, perhaps corresponding to the "glanzkörper" of the *Ascaris* sperms, may be orientated any way. It may be added that these sperms are capable of amoeboid movements. Plate 4, B, a-i, are sketches made of such moving sperms; the pseudopodial processes are only finely granulated, the larger granules resting behind; they then show no such definite arrangement as sketched in Plate 2, A.

No description of the male has been published. The sexual number is 53.1, that is, to every 100 females there are 53.1 males. (A total number of 170 specimens was examined with regard to sex.) In the single, reflexed, male sexual organ, *vesicula seminalis* and *ductus ejaculatorius* are not well set off from each other. Plate 1, G and H illustrate the curved, paired and symmetrical spicula, each with a strengthening rib and a somewhat cephalated proximal end. A

ledge which soon tapers and nearly disappears springs from the cephalated part and perhaps forms either the edge of a membranous, wing-like, lateral part of the spicula, or the cutinized part where the very inconspicuous and weakly developed *protractores spiculorum* are attached (Pl. 1, N). The copulatory muscles are indistinct and the details of the bursal muscles doubtful, a result of the small number of muscular fibrils, rather than absence of the muscles. As to the papillae typical of male *Cephalobi*, the present material shows much variation in number and location (Pl. 1, I, J, K, L, and M). The European *Cephalobus elongatus* De Man is, as Cobb remarked, perhaps identical with the present form; but it differs in the pharynx [see descriptions by De Man (7) and Marcinowski (8)] in the position of the papillae of the male; and also the position of the excretory pore is much farther cephalad. De Man (7) shows the gubernaculum of *elongatus* linear, states its great variability, but omits morphological details. Thus the relationship of the two forms is uncertain, but the variability, with transgressional characters of both, suggests identity. The following formulae indicate much variation in the length of the individuals and of the organs:

female	1.4 1.3	14. 3.1	21. 3.7	'58. 4.6	93.4 2.5	0.935 (0.7-1.4)mm. (11 specimens measured).
male	1.1 1.3	15. 3.1	20. 3.3	'M 3.5	94.1 2.7	0.863 (0.74-1.08)mm. (9 specimens measured).
Females						
$\alpha$ -21.9 (19.6-29.4)						
$\beta$ -4.8 (4.34-5.88)						
$\psi$ -15.1 (11.8-25.0)						
} n-11.						
Males						
$\alpha$ -28.0 (27.0-29.4)						
$\beta$ -4.9 (4.6-5.1)						
$\psi$ -11.2 (14.9-20.0)						
} n-9.						

The largest specimens were from cultures on concave slides in a drop of water containing parts of grains, leaves, and stalks of corn (*Zea mays*); various other plant materials were also used with success.

Cobb succeeded in rearing *subelongatus* in macerated silage; in this case cockroach meat was also used, both foods being productive of good results. *Subelongatus* may therefore be regarded as omnivorous and well fitted for varied life conditions. More attention should be given to the ability of the genus *Cephalobus* to enter and live in healthy plants; hitherto the inclination has been to disregard *Cephalobi* as the cause of injury to plants.

KIGELIA PINNATA, THE AFRICAN SAUSAGE TREE, AS A HOST PLANT OF HETERODERA RADICICOLA

A knowledge of plants subject to root-knot, caused by *H. radiculicola*, is of importance to horticulture, agriculture, and forestry.

*Kigelia pinnata*, the sausage tree, must be added to the list of host plants of *H. radiculicola*, since young plants, apparently soil-infested, raised from seed and brought by Mr. Woods of the inspection office of the Federal Horticultural Board, showed attacks of this parasite. It may also be noted that snapdragon (*Antirrhinum majus*) plants from Washington, D. C., were badly infested by *H. radiculicola*.

REMARKS ON THE CHRYSANTHEMUM NEMA, APHELENCHUS RITZEMABOSI SCHWARTZ, AND ITS PRESENCE IN THE UNITED STATES

The presence of this nema pest in the United States has not yet been definitely established. Atkinson (1), mentioned an *Aphelenchus* sp. causing disease in chrysanthemum buds from Baltimore, Md.

In August, 1923, chrysanthemum and *Phlox drummondii* from Washington, submitted by Florence Hedges, were found infested by *Aphelenchus ritzema-bosi*, as were also chrysanthemums sent later from Quaker Neck, Chestertown, Md. The chrysanthemums showed lesions similar to those mentioned by Osterwalder (14), that is, the leaves partly yellow, or yellow and brown. The phlox leaves were mostly dried or yellow, very few being green. Nemas were numerous in the chrysanthemum leaves, but few in the phlox, where they were found in the leaf blades and stem. Although there are Aphelenchi said to infest different plants, some closely related to, some synonymous with *ritzema-bosi*, comparative examination of many specimens from all these host plants is necessary before a final conclusion is reached. Opinion on the views of Ritzema-Bos (17), Marcinowski (9), Molz (12), Schwartz (19), Stewart (21) and Goodey (6) on the taxonomic position of these nemas is, therefore, withheld.

The species here mentioned most closely resembles *A. ritzema-bosi* Schwartz, hitherto known only from chrysanthemums. As Goodey recently stated (6), this form is undoubtedly synonymous with Stewart's *A. phyllophagus*; also the specimens from South Africa mentioned by Sandground (18) as *A. phyllophagus* Stewart, belong to *A. ritzema-bosi*.

In August, 1923, Dr. Sandground sent the writer *A. ritzema-bosi* in dried chrysanthemum leaves kept since February, 1921. They were partly revived in water. Only larval specimens of the stage before the last moult revived, the first specimens reviving after 18 hours and soon moulting. The present species, therefore, can stand desiccation for at least 22 months. When again dried for from 2 to 5 weeks, they revived once more in a much shorter time—10 to 12 hours. Specimens which moulted after 22 months of dormancy withstood afterwards a drying of 3 weeks in the adult stage.

The specimens from Washington and Chestertown had fine annules and two sublateral wings on each side. The head seen laterally was similar to that drawn by Schwartz (19, p. 312, fig. 12). The excretory pore, on which Stewart lays much stress (21), was rather variable in position; in some specimens scarcely the length of the oesophageal bulb behind this latter and rather in front of the nerve-ring; in others, well behind the nerve-ring. The male tail end resembles that figured and described by Schwartz (19) and Stewart (21) for *A. ritzema-bosi*, except some specimens that show a papilla near the middle of the ventral side and just in front of the very end (Pl. 2, B). In other specimens this papilla was scarcely seen or not at all; in which case no true papilla was formed, but only a fine tube. It is uncertain whether this was the outlet of a gland, no glandular cells being seen to be connected with it. Therefore, it is believed that the present species has really two ventro-median postanal papillae in the male, the most inconspicuous one being near the tail end. In some specimens the papillae are easily seen with only a low magnification, and at first the writer thought it to be *A. helophilus* de Man as figured by Kati Marcinowski (9). Plate 2, B shows a lateral view of such a male tail end. The writer has no additional information as to the effect on the host. The observation of former investigators that the nemas leave the plants when put in water can be confirmed.

#### REMARKS ON DORYLAIMUS REGIUS DE MAN AND ITS FOOD

Rather large-sized species of the nema family Dorylaimidae are numerous in most soils; hence the question of their economic significance and relationship arises. On account of the spear-like onchium and sucking apparatus, a more or less parasitic relationship to plants has been pointed out by investigators. In common with various other nematologists, the writer has observed Dorylaimi with green intestinal contents, in certain cases recognizable as chlorophyll.



Thus, the belief that some Dorylaimidae feed partly on plants seems well founded; however, this is apparently not true for all species. It is doubtful whether the Dorylaimus species, so numerous at great depths in fresh-water lakes, live on plants; as plants seem to be unavailable there, a carnivorous feeding habit is more probable. Many soil-inhabiting species of Dorylaimus and related genera also may be carnivorous.

The following study of the intestinal contents of *Dorylaimus regius* de Man indicates that at least this species seems to be carnivorous. September, 1923, six *Dorylaimus regius* De Man (four females; two males) were submitted by the Plant Quarantine Inspection Service for identification. Four, found by R. G. Cogswell, of Philadelphia, Pa., were about roots of *Amaryllis* imported from Germany, and two about roots of roses from Norway. A study of these enables the writer to add to the knowledge of this rather uncommon species. Measurements, Cobb's formula:

	0.	6.1	15.	12.5	11.0	
female	0.5	1.5	1.6	2.1	1.5	6.919 mm.
male	0.	?	17.1	-M-	98.6	
	0.5	?	1.9	2.1	1.6	5.848 mm.

De Man's formula:

Female+1	Female+2	Male
$\alpha$ -40.5	47.9	47.1
$\beta$ -6.18	6.4	5.7
$\psi$ -93.5	100.7	86.0
$v$ -52.3%	51.3%	

The two Norwegian specimens (females) differed chiefly in the length of the tail, which was much shorter and showed measurements as follows:

	Female 1		Female 2
Total length	- 5.686 mm.	Total length	7.820mm.
$\alpha$ -	38.0		51.4
$\beta$ -	6.08		6.2
$\psi$ -	133.8		154.3
$v$ -	50.5		47.0

The typical *Dorylaimus regius* De Man had hitherto been found only by De Man (7), Brakenhoff (2), and Marcinowski (8). Micoletzky (11) considers *D. superbis* de Man to be a small variety. Perhaps also *D. eurydoris* Ditlevsen belongs to *D. regius* (5).

The cuticle has at least three layers (Pl. 3, F); the surface layer shows a transverse striation similar to the lines of a finger-print (Pl. 3, G), and sometimes a faint indication of cross fibers, as in many Mermithids. The number of cephalic papillae seems greater than hitherto described; the lateral lips have only two papillae, but each submedial lip has three (Pl. 2, C, D, E, F, and G). The papillae of the second circlet are double on the submedial lips. Another organ hitherto not mentioned for the present form is the amphid (lateral organ). The whole lip region apparently can be protruded or retracted, and the amphids are accordingly seen more or less well. In Plate 2, E, the lip region is somewhat protruded and the amphid is partly exerted; whereas in Plate 2, C and D the whole organ, hidden inside the ridge just behind the lip region is disclosed as a broad pouch-like cavity with a great number of bent and curved terminals (Endfasern) as in a few other Dorylaimidae studied. The wall of this pouch-like structure is thicker in the specimen in Plate 2, C and D than in that of E. As Plate 2, F shows, the pouch is somewhat divided into an anterior and a posterior part, and at its base enters what the writer regards as the "amphidial" nerve with its numerous fibers, surrounded by a large cell of apparently glandular character, which can be followed a distance towards the nerve-ring. Plate 2, G shows the amphids of the specimens of Plate 2, C and D, in a front view.

As described by Brakenhoff (2), papillae more numerous ventrally than dorsally, can be observed along the entire body in the lateral and submedial regions. The writer believes them to be rather of nerve character than outlets of glands as Brakenhoff suggests (Pl. 4, A; Pl. 2, C and D; and Pl. 3, A, B, C, and D). There seems to be a rudimentary excretory pore ventrad from the nerve-ring (Pl. 4, A), and several glandular cells at the cardia, but apparently all on the dorsal side of the oesophagus and intestine.

Plate 3, C and D show that the specimens from Germany (Pl. 3, C) had a longer, less blunt tail than those from Norway. In both males and females, papillae similar to the body papillae could be seen on the tail end, their position differing in each specimen. At the very end of the tail the cuticle showed in one specimen (Pl. 3, A and B) a tenon-like element of somewhat darker color, not quite reaching the terminal surface. Another had the same region radially striated (Pl. 3, C), the rest of the animals showing none of these structures. Therefore, these and similar features, as they occur in other *Dorylaimus* species, are probably of little taxonomic value.

Since only a single male has so far been found, or possibly two (if Ditlevsen's *D. eurydoris* belongs to the present species), some information regarding it may be added. The spicula are sketched in Plate 3, A and B. There are two gubernacula; what probably are details of their actuating muscles are also figured. Most interesting are the papillae of the male, which differ in the two present specimens (Pl. 3, B and L). In one specimen the series of preanal, contiguous papillae, beginning somewhat in front of the spicula was regular and continuous (Pl. 3, B), while in the other specimen the posterior papillae of the series were separated from the others by a long interval. The first specimen in all respects resembles the male *D. eurydoris* as described by Ditlevsen (5). Brakenhoff's male (2) has two additional papillae and the series of papillae begins directly opposite the proximal ends of the spicula.

#### ECONOMIC SIGNIFICANCE AND FOOD OF DORYLAIMUS REGIUS

In estimating the economic significance of soil nemas, a knowledge of their food and feeding habits, of which very little is known, is of chief importance. There are two ways to determine the food of these nemas, (1) by experimental feeding, (2) by a study of the intestinal contents. The first is difficult on account of the environment—the soil; the second, because the sucking way in which most forms feed renders the intestinal contents more or less unrecognizable; nevertheless, knowledge important to the study of soil economy can be gained by careful study of the intestinal contents. Such a study of the four specimens of *D. regius* resulted in finding an oligochaete seta (Pl. 3, J); there is figured at the right of the bristle, at the same magnification, the spear of the *Dorylaimus*, to show that the entrance of the bristle through the spear was possible. In another specimen the intestine was filled with a gray mass containing many nuclei of such a nature that hardly anything other than the gonad of an animal can be considered the source of it. Therefore, *D. regius* undoubtedly ingests animal matter. In an earlier paper Steiner (20) called attention to the apparent relationship of the Mermithidae to the Dorylaimidae, these latter being postulated as the ancestors of the former. The fact that certain Dorylaimidae actually feed on animal food is clearly in harmony with this view.

#### PARATYLENCHUS NANUS COBB INFESTING THE ROOTS OF ZINNIA ELEGANS

*Paratylenchus nanus* Cobb (4) is perhaps synonymous with *P. bukowiensis* Micoletzky (11, p. 606). Cobb found his specimens about the roots of grass, Devil's Lake, N. Dak., and at Four Mile Run, Falls Church, Va. Micoletzky

had a single specimen from roots of grasses from a sandy meadow in the inundation of the Pruth River, near Czernowitz, Bukowina.

The present specimens were taken from the exterior tissues of roots of *Zinnia elegans* sent by Mary C. Brooks, Quaker Neck, Chestertown. The nemas, numerous where the surface of the root was brownish, occurred as single isolated specimens spread through the tissues. Although more than 200 specimens were closely examined, no males were found. The writer can, however, confirm the statement of Cobb that spermatozoa are present in the female uterus and that therefore the species must be considered syngonic. The roots showed no abnormalities other than the above-mentioned brownish surface spots, no swellings, etc., indicating that plants react very differently to various parasitic nemas. This seems to the writer to be additional evidence that the chief damage Heterodera, some Tylenchi and Aphelenchi do to their plant hosts is caused by the secretion of toxic nemic substances. Perhaps different plant species, and even different varieties of the same species, vary greatly in their susceptibility to these toxins, and consequently the size or number of the deformations of plants may not be an index to the number of parasitic nemas living therein.

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## PLATE 1

A.—*Cephalobus subelongatus* Cobb: head end sublateral view; amph, amphid.

B.—Same; head end: m inc, main incision between main lips.

C.—Same; one main subventral lip with the two papillae separated by a flat incision; m lb, main lip; subv ppl, subventral papilla; lat ppl, lateral papilla behind the amphid.

D.—Same; cardiac region of the body; cls, cells which apparently belong to the excretory apparatus; nrv r, nerve ring; p ex, excretory pore; ren, renette or ventral gland; t p, transparent tissue of the cardiac region of the intestine; valv, the ribbed valvulae of the valvular apparatus; valv el 2, second group of three rod-like valvular elements; valv el 3, third group of three valvular elements of same form as those of group two.

E.—Same; front view of the head end; amph, amphid; sub p, submedial papilla; lat p, lateral papilla.

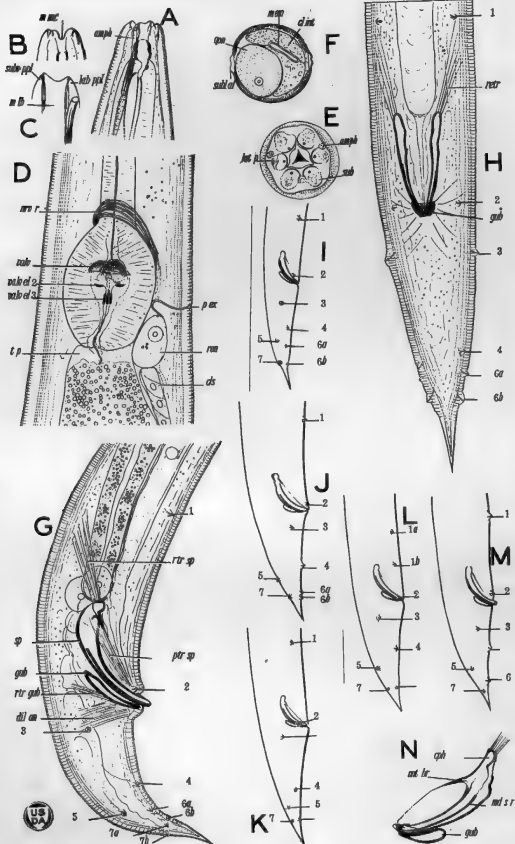
F.—Same; cross section through the middle region of the body; cl int, intestinal cell; mem, cutinized membrane around intestinal cavity; gon, gonad; subl al, sublateral wing.

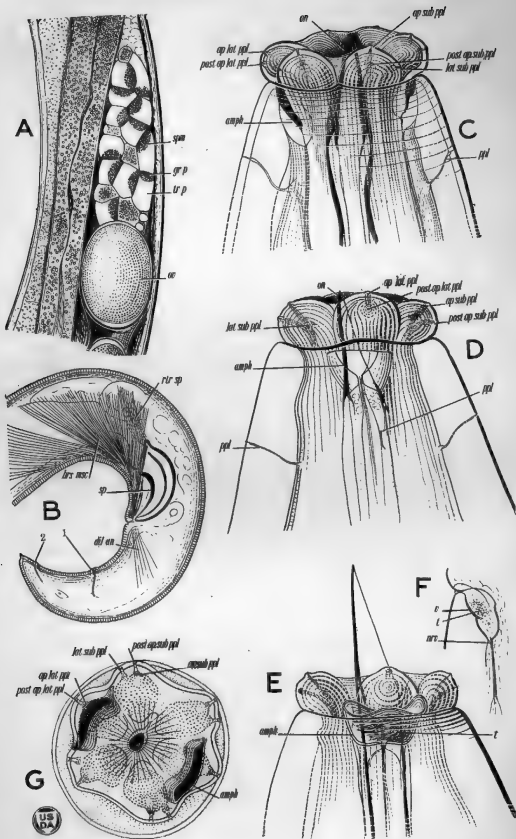
G.—Same; tail end of a male; dil an, dilatator muscle of anus; gub, gubernaculum; rtr sp, retractor spiculi; ptr. sp, protractor spiculi; sp, spiculum; 1-7, papillae of the male tail end; some double, as 6a, 6b, 7a, 7b.

H.—Same; ventral view of the male tail end; gub, gubernaculum; retr, retractor spiculi; 1-6, papillae of the male tail end; number 6 is double, number 7 can not be seen as situated on the dorsal side.

I, J, K, L, and M.—Same; sketches of different male tail ends to show the variations in the number and position of the papillae, of which some are double.

N.—Same; a lateral view of the spiculum and the gubernaculum; cph, cephalated proximal end of spiculum; gub, gubernaculum; md s r, median strengthening rib of spiculum; vnt br, branch of the ventral rib.





## PLATE 2

A.—*Cephalobus subelongatus* Cobb, region of female body with uterus filled with spermatozoa and eggs; gr p, granular part of a spermatozoön; ov, egg; trap, transparent part of a spermatozoön; spm, a spermatozoön.

B.—*Aphelenchus ritzema-bosi* Schwartz; tail end of a male in lateral view; brs msc, bursal muscle; dil an, dilatator muscle of anus; rtr sp, retractor muscle of spiculum; 1-2 papillae.

C.—*Dorylaimus regius* de Man; median view of the head end; amph, amphid; ap lat ppl, apical lateral papilla; ap sub ppl, apical submedian papilla; lat sub ppl, lateral submedian papilla; on, spear; post ap lat ppl, postapical lateral papilla; post ap sub ppl, postapical submedian papilla; ppl, papilla of the body surface.

D.—Same; lateral view of head end; lettering the same as in C.

E.—Same; lateral view of a head end with the opening of the amphid (amph) protruded and free; t, terminals.

F.—Same; optical section through the amphid in profile position; nrv, amphidial nerve; t, sections through terminals; v, amphidial wall.

G.—Same; front view of head end; lettering same as in C.



### PLATE 3

A.—*Dorylaimus regius* de Man; ventral view of the tail end of a male; an ppl, anal papillae; d cut, differentiated cuticular tissue; gub, gubernaculum; ppl, ventromedian papillae; sp, spiculum; s ppl, skin papillae.

B.—Same; tail end of a male in lateral view; an ppl, anal papillae; brs msc, bursal muscles; d cut, differentiated cuticular tissue; gub, gubernaculum; ppl, series of preanal papillae; ptr sp, protractores spiculorum; rtr sp, retractor spiculi; 1-4 skin papillae.

C.—Same; tail end of a female from Germany; dil an, dilatator muscle of anus; ppl, skin papilla; str cut, radial striation of the cuticle.

D.—Same; tail end of a female from Norway; compare with C and note the much shorter tail.

E.—Same; transverse striation of the surface of the cuticle.

F.—Same; optical longitudinal section through the skin; hyp, hypodermis; 1-3 different layers of the cuticle.

G.—Same; one of the ventromedian preanal papillae.

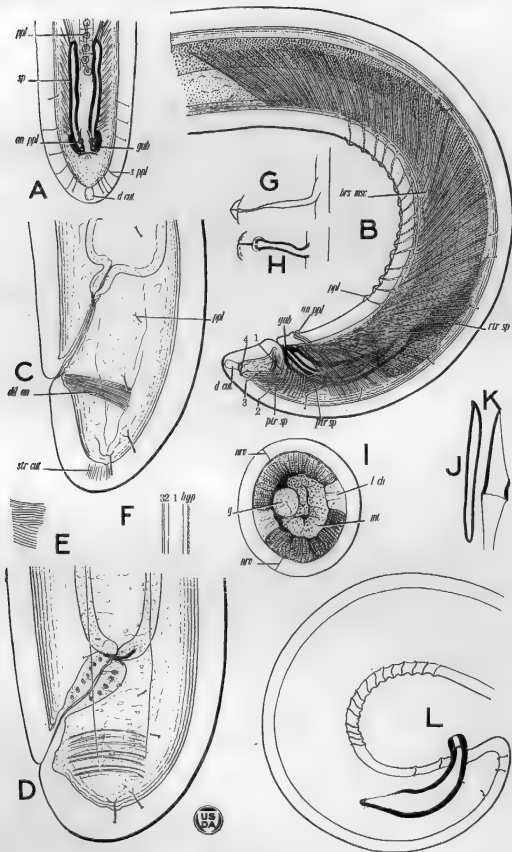
H.—Same; one of the anal papillae; compare the difference with G.

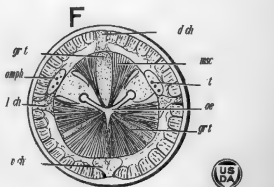
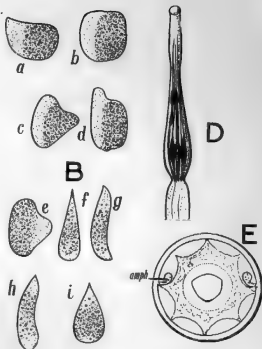
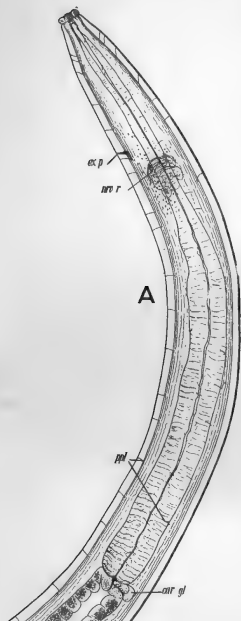
I.—Same; cross section through the middle region of the body; g, gonad; int, intestine; l ch, lateral chord; nrv, skin nerve.

J.—Sketch of a bristle of an oligochaete as found in the intestine of *Dorylaimus regius*.

K.—*Dorylaimus regius* de Man; the spear drawn with the same magnification as J to show the possibility of the entering of the bristle through its lumen.

L.—Same; tail end of a male with a different order of the ventromedian preanal papillae.





#### PLATE 4

A.—*Dorylaimus regius* de Man; head end of the body with beginning of the intestine; car gl, cardiac gland; ex p, excretory pore; nr v r, nerve ring; ppl, skin papillae.

B.—*Cephalobus subelongatus* Cobb; a-i, sketches of spermatozooids showing amoeboid movements.

C.—Same; dorsal view of head end; amph op, amphidial opening; d l, dextero dorso submedial lip; gl, apparently glandular cell attached to the amphidial pouch; t, terminals (sensilles?).

D.—Same; amphid seen laterally.

E.—Same; optical cross section of the head just behind the lip region at the level of the amphidial openings; amph, amphid.

F.—Same; cross section behind the pharynx; amph, amphid; d ch, dorsal chord; gr t, granular tissue; l ch, lateral chord; msc, radial oesophageal muscles; oe, oesophageal channel; t, terminals, v ch, ventral chord.

# THE INFLUENCE OF ENVIRONMENT ON SEX IN HEMP, *CANNABIS SATIVA* L.<sup>1</sup>

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## INTRODUCTION

Since environment apparently has such a potent influence on sexual expression in hemp, *Cannabis sativa* L., the writer found it necessary during the course of an investigation of the genetics of sex in this species, to study the effect of relative length of day and night upon vegetative and floral development. The problems connected with this phase of the work have been pursued only as far as was necessary to enable the major work to be carried on and the results are not to be considered as final from the physiological standpoint. But since the work has been discontinued it seems desirable to report the results that they may be available to others who are interested in problems of this kind.

Physiological studies similar to those reported here have been pursued by several investigators, especially Garner and Allard (1, 2, 3).<sup>2</sup> Since they have reviewed the literature which pertains to this phase of physiology, it seems unnecessary to attempt further review here except to mention Schaffner's work on hemp. Schaffner (5, 6, 7) found that apparently it is possible to control sex in hemp. When grown during the long days of summer the sexes remain pure and the ratio of staminate to carpellate plants is approximately 1:1. When grown in the greenhouse during the short days of winter, however, both carpellate and staminate plants showed reversal to the opposite sex. Furthermore, the proportion of plants which showed sex reversal seemed to be roughly proportional to the length of the darkness period. In the shortest days of winter some of the plants showed almost 100 per cent of intersex plants. Schaffner therefore maintains that sex in hemp is non-Mendelian in nature and is under the control of environmental factors.

## SEXUAL DIMORPHISM

When grown under normal conditions hemp is when mature dimorphic in both vegetative and flower characters. When grown in the greenhouse during the winter the dimorphism is even more pronounced. The principal sexual differences are as follows:

**Staminate plant:** More slender and taller than the carpellate plant because of the rapid elongation of the internodes just prior to anthesis; terminal inflorescences with practically no leaves; flowers normally with five sepals and as many anthers; much shorter life than the carpellate type (Pl. 1).

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<sup>1</sup> Received for publication March 26, 1924—issued November, 1924. The results reported in this paper were obtained at the Laboratory of Plant Genetics, Bussey Institution of Harvard University, and completed in the U. S. Department of Agriculture.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 1080.

**Carpellate plant:** More vigorous but shorter than the staminate type; terminal inflorescence leafy; broad crown of leaves; flowers with perianth but no vestiges of stamens; weight at maturity about twice that of the staminate type; longer life.

Probably hemp is as dioecious as any of the so-called dioecious species but it is a well-known fact that certain individual plants sometimes bear both staminate and carpellate flowers at the same time, and that such intersex types appear in larger numbers when the daily exposure to light is short, such as prevails in northern latitudes during the winter months. The vegetative differences, however, are the same for the plants which remain sexually pure and for those which develop some flowers of the opposite sex. That is, a change in the sex of the flowers produced is not accompanied by a corresponding change in the vegetative characters. In other words, the dimorphic vegetative types occur in approximately a 1:1 ratio. It seems quite clear that probably the vegetative type of the plant was already determined in the embryo from which the plant grew but that the sex of each flower was not determined until a somewhat later stage. The natural thing is for each vegetative type to produce flowers of the sex characteristic of that type but so far as any differences which exist in the structural elements of the two types are concerned there is no reason why both sexes of flowers can not be borne by both staminate and carpellate plants. The common occurrence of intersex types in the greenhouse shows that this is true. The writer has several times grafted staminate tops on carpellate stocks and vice versa without any effect upon the sex of the flowers, but does not mean to say that pollen which is produced by a carpellate type plant is the same genetically as that produced by a staminate plant. But since this paper is concerned only with the effect of environmental factors, a discussion of the hereditary aspects of the case will be deferred until a later paper.

#### PRELIMINARY OBSERVATIONS

The writer's attention was first directed to the effect of relative length of day and night upon sex in hemp by the peculiar behavior of several specimens which were being grown in the greenhouse during the late winter and early spring months. The seed was planted about the first of December and flower buds were first distinguishable on January 4. Relatively few flowers were produced and many of these were so abnormal that few seeds were matured. Following the maturity of this crop of seed the plants remained quiescent for several weeks and then resumed growth during the late spring months flowering again at about the normal blooming time for hemp which is grown in the open. It was thus possible to cause two crops of flowers to be produced in one year. The flowers which were produced during the second flowering period were normal and a fair crop of seed was matured. The same general effects are produced when hemp which has been planted in the open is moved into the greenhouse in the fall. The writer moved several such plants into the greenhouse before killing frosts occurred to ascertain if they could be held over in a semidormant condition. These plants dropped most of their leaves and made no additional growth for several weeks. During the month of December new leaves appeared and a small number of flowers were produced, most of which were abnormal. The production of flowers was still in progress when the plants were discarded late in January. This case refers only to carpellate forms, since the staminate plants die in late summer and apparently can not be kept alive by moving into the greenhouse.

The sexual abnormalities which appear during the short winter days are of the diverse kinds. The most common type among the plants has been the production of intersex flowers on plants of the staminate type. The individual flowers in

such cases vary all the way from pure staminate to pure carpellate. The writer has observed flowers containing two anthers and a rudimentary ovulary with one, two, or three stigmas; an apparently normal ovulary and two or three rudimentary anthers; three normal stamens and one terminated with a stigma; two ovaries, each with three stigmas; and many others. Of course, many of such abnormal flowers are not functional and therefore the highly intersex types seldom produce much pollen and set few seeds. Such behavior in the greenhouse during the winter when the daily period of light is relatively short indicates, as Schaffner (6) has already shown, that sex in hemp is largely under the control of environmental factors. If this is true it should be possible to obtain almost any desired degree of "sex reversal" by regulating the environmental factors.

#### PLAN OF THE EXPERIMENTS

For the purpose of producing different lengths of daily exposure to light, a series of ventilated chambers were used from which light could be excluded at will by means of sliding sashes upon a glass roof. The walls of each chamber were covered with black paper to prevent any light which might gain entrance from being reflected. Observation showed that when the shutters were closed these rooms were dark even at noon and gave a sufficiently good imitation of night for the purposes of this experiment. In the discussions which follow these chambers will be spoken of as the dark house. With such an arrangement, the large amount of labor connected with the daily moving of plants in and out of the house was eliminated and the effects of adverse weather conditions, such as high winds and rain, were reduced to a minimum.

The maximum difference in the temperature of the rooms did not exceed 3° C. during the course of the experiment. Although the difference between the temperature of the outside air and that inside the house was much greater than this, such differences are not of importance to this investigation, since adequate control plants were grown in one of the rooms. In work where the control plants were grown in the open it would, of course, be necessary to take into consideration the difference in temperature of the inside and the outside air.

The plants for this experiment were obtained from seed sown in flats in the greenhouse. As soon as the seedlings were well up, the flats were removed to the dark house and the different exposures to light were started at this time and continued until the maturity of the plants. At the time of potting, all of the seedlings were saved.

Inasmuch as soil and size of the pot have a marked effect on plant growth, great care was taken to keep these two factors constant for all lots of seedlings. Sufficient soil was thoroughly mixed at the start to last throughout the experiment. The potting of all lots was always done on the same day so that the plants in each house would have the same chance in this respect. The seedlings were first placed in 2-inch, later in 3-inch, and finally in 5-inch flower pots. To further prevent differences due to nutrition, all pots were placed on wooden benches so that the roots could not go through the bottom of the pots into soil.

Water was applied during the course of the experiment in such quantities as to keep the moisture content of the soil practically the same in all pots. Moisture tests were not made because it was believed that fluctuations in water supply within the limits present in this experiment have no effect upon sex in hemp.

The lengths of exposure used were 5 hours, 7 hours, 10 hours, and the normal length of day for this latitude. By reference to figure 1 it is seen that the period from sunrise to sunset during the course of the experiment was from 12 to slightly over 15 hours daily. The sashes were removed from all compartments at 7 a. m. and replaced as follows: In the 5-hour exposure at 12 m., in the 7-hour exposure

at 2 p. m., and in the 10-hour exposure as 5 p. m. Those which were given the full day exposure were treated in the same manner as the other plants except that they were not darkened.

In mentioning sex ratios the number of carpellate to 100 staminate plants has been used throughout this paper.

The effects produced by the various lengths of exposure to light are shown by the summary which is given in Table I.

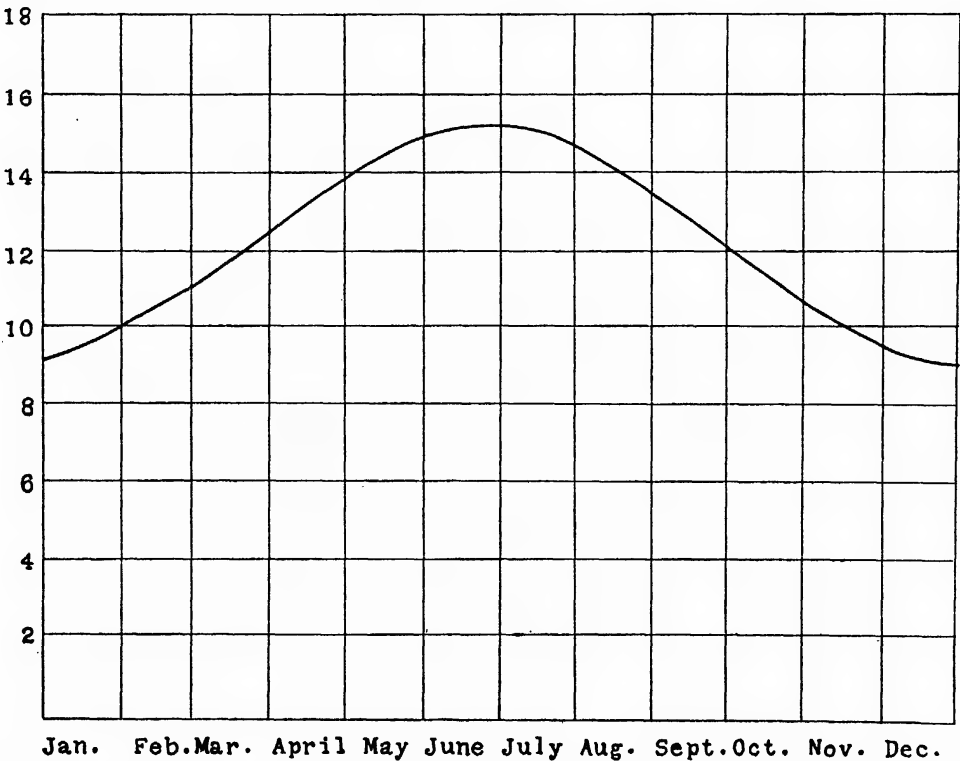


FIG. 1.—Graph showing the changes in length of day during the year in the latitude of Boston, Mass. Ordinates indicate 2-hour intervals of the day and abscissae indicate monthly periods of the year

TABLE I.—*Effect of day length on growth and reproduction*

Day length	Date planted	Budded	Sex ratio	Total number	Number died	Height
						Meters
5 hours.....	May 13	June 21	128:100	128	19	0.21
7 hours.....	do.....	June 14	119:100	101	20	0.50
10 hours.....	do.....	June 15	111:100	95	11	0.75
Day—inside.....	do.....	Aug. 13	115:100	73	23	2.05
Day—outside.....	do.....	July 14	140:100	120	4	1.04
Day—in plat.....	do.....	Aug. 14	97:100	133	-----	3.38
Total.....	-----	-----	117:100	650	82	-----

BEHAVIOR OF THE PLANTS

FIVE-HOUR EXPOSURE.—Plants which were exposed to light for only 5 hours daily showed very little development of either the vegetative or the reproductive parts. Side branches were absent and the leaves were few in number, small, and yellowish green in color. The number of flowers produced was very small and the maximum crop of seed matured by single carpellate plants was not over 12. Flower buds first appeared 38 days from the date of planting. The



sex ratio was 128:100 in a population of 128. At maturity the mean height of the entire lot was only 0.21 meter and the majority of the plants had to be supported. It is evident that this length of day approaches the lower limit in which hemp can be grown to the flowering stage.

**SEVEN-HOUR EXPOSURE.**—An increase of 2 hours daily over the 5-hour exposure not only caused the plants to develop buds 7 days sooner but also caused a significant increase in the development of vegetative and reproductive parts. The mean height attained was 0.50 meter and the stems were sufficiently strong to stand without support. The sex ratio was 119:100.

**TEN-HOUR EXPOSURE.**—The chief difference shown by this lot of plants was one of greater development. The mean height at maturity was 0.75 meter, leaves were large and deep green in color, and flowers were very abundant. Buds appeared 33 days from date of planting which is practically the same length of time required by plants in the 7-hour exposure to develop buds. The sex ratio was 111:100.

**FULL DAY IN DARK-HOUSE.**—The lot which was grown in the dark-house but exposed to the normal length of day for this latitude made so much growth that the size of the pot probably was a limiting factor in their development. The mean height at maturity was 2.05 meters. The main stem did not develop proportionally in thickness but remained slender and many of the plants were unable to support themselves at the time of flowering. The leaves were large and numerous but side branches were not produced to any extent. The time of flowering was greatly delayed in this lot by the long daily exposure to light, buds first appearing 84 days from the date of planting. The sex ratio was 115:100 in a population of 73.

**FULL DAY OUTSIDE IN POTS.**—A number of plants were grown in 5-inch pots in the open as a control on the lot which was grown in the dark-house. Flower buds appeared in 80 days from date of planting. Due to inability to properly control the moisture content of the soil, growth was somewhat irregular and the results obtained with this lot are not comparable with the results obtained with the other lots. The mean height at maturity was 1.04 meters and the sex ratio 140:100 in a population of 120.

**PLANTED IN THE GARDEN PLAT.**—Hemp which was grown in the garden plat attained a much larger size than did any of the potted plants. The mean height at maturity was 3.38 meters, although individual specimens reached a height of over 4.5 meters and a main stem diameter of over 1 inch. Side branches were produced in large numbers and flower production was very profuse. It is a significant fact that although this lot made a much greater vegetative development, they produced flower buds at the same time as did the lot which was grown in full-day exposure in the dark-house. This result indicates that the differences in development are due to factors other than light and that the time of flowering is not determined by the extent of vegetative development. The sex ratio of the plants in the garden plat was 97:100, the nearest to unity of any of the lots, but of course this is probably only a matter of coincidence.

#### EFFECT OF LENGTH OF EXPOSURE TO LIGHT ON GROWTH

The results show that both the rate and extent of growth, as well as the time of flowering, are greatly affected by the length of daily exposure to light. The effect on vegetative and sexual development can best be discussed separately.

TABLE II.—*Effect of length of exposure to light on growth*

Exposure	Date planted	Height	
		July 6	Aug. 15
5 hours.....	May 13	<i>Meter</i> 0.22	<i>Meters</i> 0.47
7 hours.....	do.....	.46	.67
10 hours.....	do.....	.65	.90
Full day.....	do.....	.71	2.28

## EFFECT ON VEGETATIVE DEVELOPMENT

Although exact measurements of the details of vegetative development in the dark-house were not taken, the mean heights give a certain measure of the effect of day length on the rate and the extent of growth. Plants in the 5-hour exposure group grew slowly at first which accounts for the small amount of growth made by the time of the first measurements on July 6. The data show that the extent of growth in all lots is roughly proportional to the duration of daily exposure to light. This does not mean, of course, that the rate of growth is proportional to the length of the daily exposure to light. The second series of measurements which were taken on August 15, show the same general effect of day length on height as the first measurements; the plants in the 5-hour day had the lowest mean height and those in the full daylight had the greatest mean height. Although it is realized that such results are not critical, they are very suggestive and agree with the work of Garner and Allard (3) who found that the increase in height of soy beans is proportional to the length of daily exposure to light.

Since the length of the growing period was greatly reduced by the accelerating action of the short exposure to light on the reproductive processes in the case of the plants which were grown in a short day, the amount of growth made by these lots was necessarily somewhat limited. The rôles played by the rate and the extent of growth, respectively, is a question which can not be answered from the data obtained in the dark-house. Another carefully controlled experiment has yielded results which show very clearly the relation between rate and extent of growth in hemp. Nine lots of 8 plants each were exposed to light daily for 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 7 hours, 12 hours, 18 hours, and 24 hours, respectively. Healthy seedlings of about the same height were selected and the treatment begun at the time of first potting. All seedlings were dark green in color and the mean height of each lot was 5 cm. The growth of each lot is represented graphically in figure 2. The rate of growth is not constant at all times but varies considerably at different periods. All lots began with a fairly low rate which gradually increased but this increase was not the same in all lots and it is due to this differential increase in growth rate that the extent of growth is not the same in all exposures at the end of a certain length of time. The lot which received 24 hours and that which received 18 hours of light, respectively, grew at so nearly the same rate that the data are represented by one curve. Their rate of growth was high from the start and it is due to the rapid growth during the first 10 days of the experiment that the height attained is greater than that of any of the other lots. Plants which were exposed to light for 12 and 7 hours daily were correspondingly slower in attaining a growth rate comparable to that of the 24-hour lot and their mean heights at the end of 34 days was correspondingly less.

Although the extent of growth was greatest in the longer light exposures, it does not necessarily follow that this result is due to a higher rate of growth over the entire period. In fact, the data show that in 7 or 12 hours of light daily the rate of growth at certain times equaled or even exceeded the rate of the 24-hour lot. The greater height at maturity in 18 or in 24 hours of light daily exposure

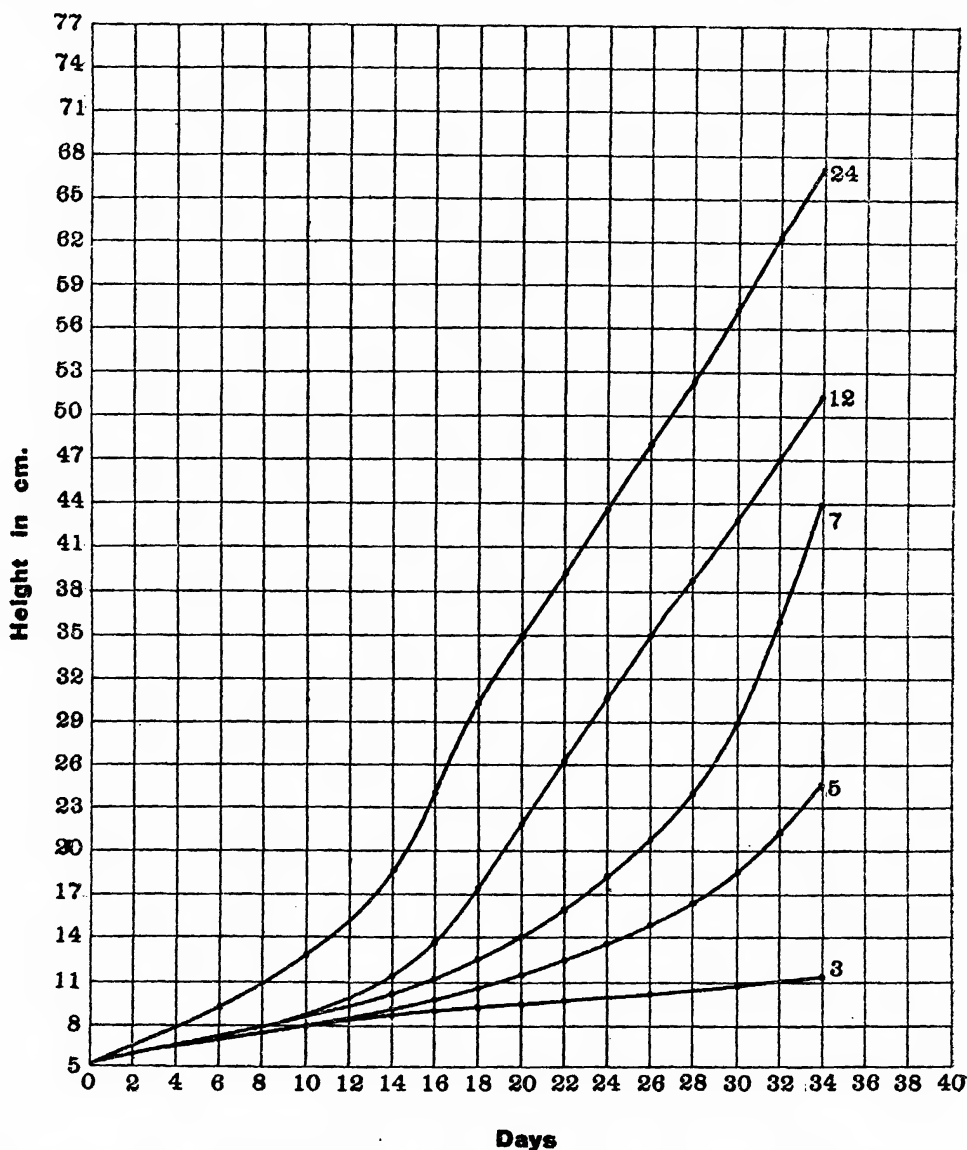


FIG. 2.—Graphs showing relation between relative length of day and night and growth in hemp. The seedlings were 5 cm. in height when the experiment was begun. Ordinates indicate growth in centimeters and the abscissas indicate the number of days. The numbers at the end of each graph show the number of hours of light received daily by each lot of plants. Measurements were made every two days

is due to the high rate during the early part of the growth period and the continuance of this rate over a long period of time. If the growth rate which the 7-hour plants have just prior to anthesis could be maintained over a long period they too would be tall but the period of this rapid rate of growth lasts but a few days and then rapidly declines to zero. Since hemp in this exposure grows relatively slowly up to the time of anthesis and then rapidly for only a short time, it necessarily follows that the plants will not attain a great height. The high growth

rate of hemp which is grown in a daily light exposure of 12 or more hours is continued up to the time of flowering, which is about 90 days from planting, and is then increased during anthesis. As a result such plants attain a relatively great height.

The minimum length of daily exposure to light required for continued growth of hemp in this experiment was 3 hours. In an exposure of 1 hour daily the plants were all dead in 20 days, and those which received light for 2 hours daily were all dead in 34 days from the start of the experiment. In these two exposures the growth was fairly rapid during the first 7 days but after this time it rapidly declined to almost zero. At the end of one week from the start these lots began to lose their rich green color and at the time of death the leaves were yellow. In 3 hours of light daily the rate of growth was low and fairly constant. Weakened development was clearly evident in the few, small, greenish yellow leaves and the very few flowers. At the end of 34 days the mean height of the lot was only 11.5 cm. as compared with 44 cm. in the 7-hour lot. It seems quite clear that it is not possible to grow hemp in a daily exposure to light which is short enough to prevent flower development and yet allow the plants to live. In this respect hemp belongs to the same class as *Cosmos* and *Bidens*.

It is unfortunate that the analysis of growth in this experiment had to be discontinued at the end of 34 days because it leaves the answers to several interesting points incomplete. The writer has not observed a difference between the growth rate of the sexes until near the time of flowering. With the approach of anthesis the staminate plants increase in height much more rapidly than the carpellate forms. After this point in the life history is reached there is therefore a differential growth rate among the sexes. Since it is impossible to determine the sexes in advance, there is no sure way of selecting equal numbers of each sex in each lot. In order to compare the growth rate from planting to maturity in different light exposures it would be necessary to determine the growth rate of each sex in each light exposure and use this information in working out a correction factor to be applied in cases where the sex ratios were not the same. So in an experiment of this kind with hemp there are two important factors to be considered—a differential growth rate of the sexes, and a second differential rate among the lots due to differences in time of flowering.

#### EFFECT OF LENGTH OF EXPOSURE ON SEXUAL DEVELOPMENT

Although marked alterations in the vegetative development were produced by various changes in the length of daily exposure to light, equally interesting, and of much greater importance to this investigation, were the effects on the reproductive processes. The length of time between planting and the appearance of flower buds increases as the length of exposure to light is increased from 7 hours daily, and an exposure of less than 7 hours daily also delays the time of blooming. The graphical representation of the data in figure 3 shows the relation between the time of flowering and the length of daily exposure to light. The criterion of time of flowering used here is that time when the sexual nature of the flower buds can be determined with certainty. The actual opening of the flowers does not occur until several days later. In 3 hours of light daily, which is the minimum for growth to maturity in hemp, flower buds appear in about 46 days from date of planting. As the length of daily exposure to light becomes greater, the time required for reaching the flowering stage becomes less until an exposure of 7 hours is reached. From this point on, the time between planting and flowering increases rapidly until it is about 90 days in a 15-hour day. Since the experiment in which hemp was exposed to continuous illumina-

tion was terminated long before the flowering stage was reached, it is not possible to say whether this lot would have bloomed sooner or later than those which were grown in the normal summer day length. The writer is inclined to believe that there would not be much difference. But with such a curve it is possible to predict the time that buds will appear with considerable accuracy, especially in the shorter exposures. Several times the writer has planted hemp when the

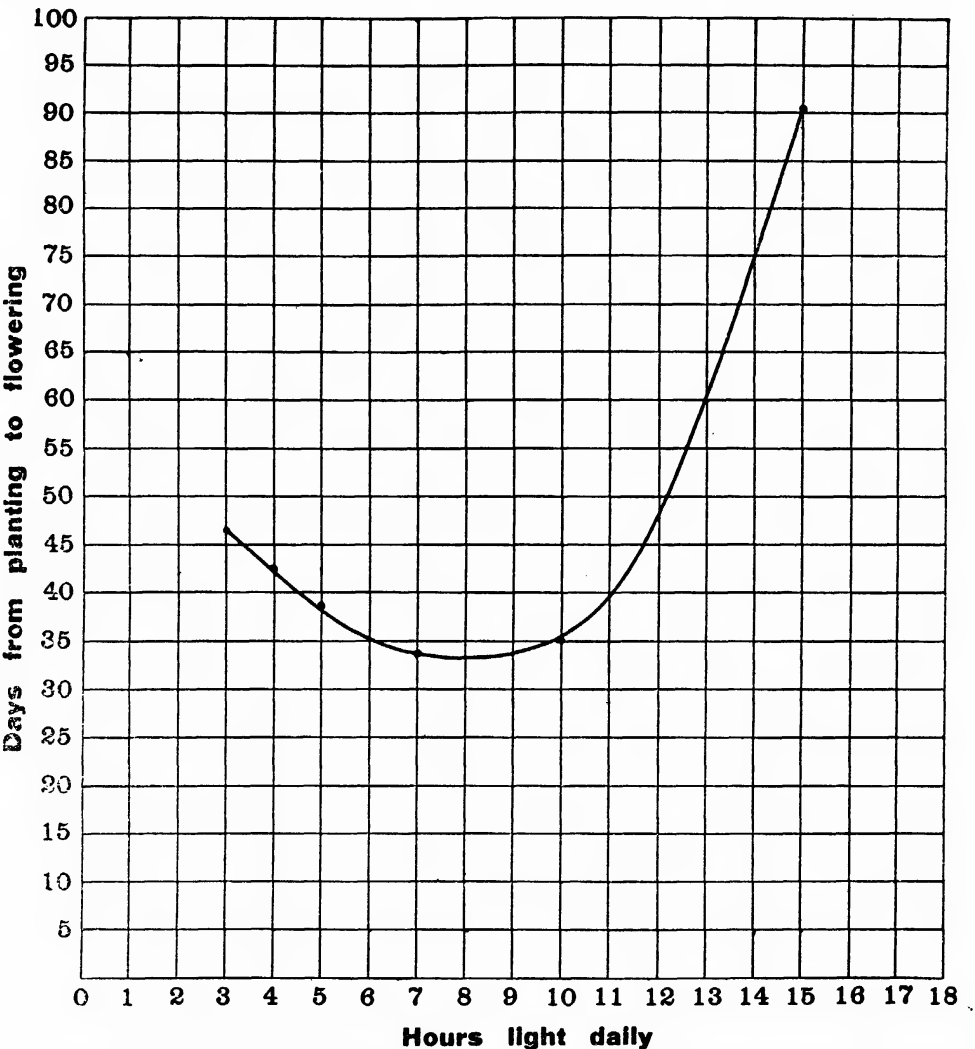


FIG. 3. Graph showing the relation between the number of days from planting to flowering and the number of hours of light received daily. Ordinates indicate 5-day intervals from the time of planting and abscissae indicate the number of hours of light received daily. Hemp can not be grown to the flowering stage in less than 3 hours of light daily

period of daily illumination was about 9 hours and has been able to predict within one day of the time when flower buds would appear.

An increase in the length of daily exposure to light after the flower buds appear has been found to greatly affect their further development. In an experiment carried on in the greenhouse during November and December it was found that flower production could be checked by increasing the period of illumination. Three plats were used. Plats two and three were given the normal daylight for that time of the year, but plat one was given, in addition, illumination from a 100-watt electric light from 4 to 8 p. m. every evening. On December 19, flower buds appeared on the plants in plats two and three. At this time the hemp in plat one

was still growing vigorously and no flower buds had appeared. Commencing on December 20, plat two was illuminated the same as plat one and in a few days the flower buds which had appeared began to show signs of being stunted. Many of these flowers never opened. All the plants in this plat were stimulated to further growth and no more flowers appeared until the period of illumination was shortened. The use of artificial illumination was discontinued on January 13, and on January 21 the hemp in plat two again showed flower buds. The appearance of buds in plat one occurred at this time also. Thus by merely giving the plants a little longer period of light each day it was possible to delay the time of flowering a month. By giving a longer exposure to light than was given in this experiment it is easily possible to delay blooming even more than this so that hemp can be made to flower in 1 month, 2 months, or 3 months from the time of planting, as the necessities of the case may require.

The differences in the sex ratios of the plants in the different day lengths may be the result of so many other causes that a serious consideration of the discrepancies from the standpoint of the effect of relative length of day and night can not be entertained. It is true that the excess of carpellate plants was greatest in the 5-hour exposure and decreased more or less regularly as the length of daily exposure to light was increased, but when the factors of death and differential germination of seed are considered, such differences cease to have any significance. If it could be assumed that all of the seedlings which died were staminate, the differences in sex ratios could be accounted for, but such an assumption does not seem justifiable here. Since the germination of the seed was only about 65 per cent, there is considerable chance that the excess of carpellate plants was caused by a differential germination of the seed. The sex-ratio differences could also be accounted for in each lot on the assumption of an unconscious selection of seedlings at the time of potting, but since all of the seedlings were saved, this factor does not affect the sex ratio of the total number of plants used in the experiment. This ratio was 117:100.

The effect of relative length of day and night upon flower development was one of extent rather than character. Plants which received less than 7 hours of light daily produced very few flowers but practically all of these were normal. This result is somewhat surprising since the appearance of hermaphrodite flowers on hemp which is grown in the greenhouse during the short days of winter has been reported by Tournois (8) and by Schaffner (5, 6, 7) who have observed that either sex may revert to the other under such conditions. Although the writer has observed similar phenomena in plants grown in the greenhouse, cases of such sexual modifications were very rare among plants grown in short days in the dark house. All the plants were examined very carefully several times but only two cases of such spontaneous "sex reversal" were found among the 650 hemp plants used in the experiment. One of these, and by far the more pronounced of the two, occurred on a staminate type which was growing in the normal length of day for that time of year. This plant produced a few hermaphrodite flowers on one of its branches. The other case was the appearance of a few abnormal staminate buds on a carpellate plant in the 10-hour exposure room. Only a few abnormal anthers were produced and these dried up before any pollen was liberated. These were the only cases of "sex reversal" among plants grown in the dark house and not otherwise treated. It seems quite clear, therefore, that a short exposure to light is not in itself the determining cause of sexual modification in hemp.

## THE EFFECT OF MUTILATIONS ON SEXUAL DEVELOPMENT

The two factors which other investigators think are the most important in bringing about a modification of sexual expression in hemp are short daily exposure to light and flower removal. Schaffner (5, 6, 7) has been able to obtain various degrees of "sex reversal" by growing hemp in the greenhouse during the short days of winter and Pritchard (4) has shown that a certain amount of modification of the sexual state is apparently caused by removing the flower buds as soon as they appear. If such factors as length of day and flower removal influence the sexual condition of hemp, it seems reasonable to suppose that the two factors combined might have a decisive influence in causing "sex reversal." To test out this assumption, several plants were selected in each of the light exposures used in the dark house and their flowers were systematically removed during the flowering period.

From 16 staminate plants in the 5-hour exposure a total of 1,832 buds were removed but only two of these plants later developed any carpellate flowers. The hemp in this room was so weak that very few flowers were produced after the operation of flower removal, so that no very definite conclusions can be drawn from this experiment. The carpellate flowers that were produced were very abnormal and occurred in hermaphrodite buds.

From 14 staminate plants in the 7-hour day a total of 5,083 buds were removed. Subsequently, all but one of these plants developed carpellate flowers of various degrees of perfection. Many of these carpellate flowers were abnormal and such forms as a three or four-styled flower, anthers terminated by a style, and mixtures of stamens and carpellate flowers in the same bud, were very common. A certain number of functional carpellate flowers were produced and several seeds were matured.

From six staminate plants in the 10-hour exposure a total of 3,555 buds were removed but only three of these plants later developed any carpellate flowers. A few of these flowers were functional and matured a few seeds. From eight carpellate plants in this day length a total of 260 flowers was removed but only one of the plants so operated on later developed any staminate buds. Since, as already mentioned, one of the carpellate plants in this room developed some staminate buds without having had its flower buds removed, the above cited case of modification of the sexual expression by mutilation does not seem important.

The removal of flowers from hemp which was grown in the open during the long days of summer was without effect in causing modifications of the sexual state. From seven staminate plants a total of 16,425 buds was removed but none of these plants later developed any carpellate flowers. If the removal of the flowers was a determining factor in causing sexual modifications in this species, as Pritchard has suggested, some of these plants should have shown a tendency to develop carpellate flowers but their behavior in this experiment indicates that the mere removal of the flowers will not necessarily cause "sex reversal." In this connection the occasional spontaneous production of normal staminate flowers by carpellate plants in the field should be mentioned. Occasionally carpellate plants of any of the varieties of hemp will produce a few perfectly normal staminate flowers. This is especially true of the Simple Leaf variety. This fact makes the occasional production of a staminate bud by a mutilated carpellate plant entirely without significance and until more positive results are recorded we can conclude that the systematic removal of flowers from hemp which is grown in the field during the long days of summer does not affect the sex of the subsequent flowers.

To obtain more evidence on sexual modification in short days, additional experiments were conducted in the greenhouse during the winter with plants

grown from seed matured in the dark-house. The classification of the plants as to sex when they first bloomed showed 164 carpellate : 162 staminate. In a few days after the first flowers opened several hermaphrodite buds were observed, the phenomenon being more common among the staminate individuals. At once the plants were separated into several lots in order to study, in detail, the effect of flower removal during the short days on sexual development.

Early in the course of the experiments it became evident that the material used was sexually different from that used by Pritchard and Schaffner in their work. They state that the tendency is apparently from femaleness to maleness but in the material used in the writer's experiments the tendency appears to be from maleness to femaleness. Only 12.8 per cent of the carpellate plants developed any staminate buds while 53.12 per cent of the staminate plants developed some carpellate flowers. The extent of the change in sex varied greatly in different plants but there can be no doubt that the changes were by far more common among the staminate type.

Although flower removal from staminate hemp plants which are grown in a short daily exposure to light does apparently cause some of them to develop carpellate flowers, there seems to be always a certain number which do not show any sexual changes. For instance, in the above experiment the flower buds were removed from 32 staminate plants in one plat, with the result that 84.3 per cent of them later produced some carpellate flowers. Only 33.3 per cent of those in the control plat showed "sex reversal." In this particular case, then, about 16 per cent of the plants showed no tendency to develop flowers of the opposite sex. Since all of the plants were treated alike, it may be assumed that they are not all genetically identical as regards the sexual state.

A similar experiment on the effect of flower removal on the sexual development of carpellate plants showed less change among the test plants than among the controls. In the plat of 32 plants grown in the greenhouse during the winter, the flower buds were systematically removed as soon as they appeared with the result that 10.53 per cent of them later produced some staminate flowers. In the control plat 14.78 per cent produced some staminate flowers. It is very evident that flower removal from pistillate plants in this experiment has not resulted in significant sexual changes.

Pruning may indirectly have some effect on development but experimental results fail to show any significant modification of sex by this means. When the top of a staminate plant is removed at the time of anthesis, or shortly before, branches arise from the leaf axils and grow to a considerable length. Sometimes varying numbers of hermaphrodite flowers appear on such branches but the results of experiment show that this is probably only a matter of coincidence. In a plat of staminate plants from which the tops had been removed 50 per cent produced some hermaphrodite flowers. In the control plat 50 per cent also showed "sex reversal." In a plat of carpellate plants from which the tops had been removed 18.1 per cent developed hermaphrodite or staminate flowers, while 20 per cent in the control plat produced some staminate flowers. It seems quite clear, therefore, that such drastic pruning as cutting off the whole top of the plant does not significantly affect the sex of the plant.

## DISCUSSION

After having observed thousands of hemp plants growing in the greenhouse and in the open, and after having tried many different kinds of stimulation in attempts to change the sex, the writer can not fully agree with the statement that the control of sex in hemp has reached a stage where it is possible to take a sample of seed and produce, at will, a stand of plants of any desired degree of sexual expression. It is true that Schaffner (6) has obtained as high as 88 per cent



of "sex reversal" in one plat which was grown in the greenhouse during the short days of winter, but when we consider that the plat contained only 16 plants, we must be careful in drawing the conclusion that the same percentage of plants in a much larger plat would show the same changes. Until it is possible to obtain the same degree of sex modification in several plats grown under the same conditions, only tentative conclusions can be drawn concerning the exact control of sexual expression in hemp. It may well be that the genetic balance in different hemp plants is such that a unit change in the environmental factors will produce more visible change in the sex of some than of others. That this balance can be upset and various sexual changes produced by varying the length of daily exposure to light there can be no doubt; but to say that any desired degree of sexual change can be obtained by this means is quite another thing.

The general term "sexual reversal" which has been used by investigators to designate a modification of sexual expression is not entirely a satisfactory one. Pritchard (4) and Schaffner (6, 7) have used the term to include all those plants which were first either pure carpellate or pure staminate, but which later produced some flowers of the opposite sex. In the majority of cases these secondary flowers are intersexual and in many cases entirely sterile. Such cases, in the strict sense of the word, are only modifications of the sex of a few flowers. A plant can not be said to have completely reversed its sex until it produces exclusively flowers of the opposite sex. It will be recognized that such a condition is rather rare in any of the so-called dioecious species. Schaffner (5, 6, 7) has undoubtedly obtained many instances of modification of the sexual expression but not many cases of "sex reversal" in the sense that the plants which were one sex at first produced nothing but flowers of the opposite sex later in their life. A few cases which approach this degree of sex change have been obtained by the writer, however. At least two of the plants which were at first apparently pure staminate, later in their lives produced nothing but carpellate flowers and matured seed. Such cases are as near a complete reversal of sex as probably can be obtained in hemp.

At the present time so little is known concerning the genetic complex of dioecious species of plants that it is not easy to draw conclusions concerning the behavior of the various intersex types. It is known that sex in hemp is somehow influenced by the environment, especially by the relative length of day and night, and also known that the time of flowering of this species is almost entirely controlled, within certain limits, by this same factor. But since the dimorphic vegetative types occur in about equal numbers regardless of the environment there must be something genetic concerned and the fact that one or both of these types later in life produce flowers of the opposite sex, is not necessarily a valid argument that genetic factors are not concerned. Many characters which are genetic in nature are affected by various environmental factors and there is no reason why sex can not be affected likewise. But since the genetics of this particular case is to be published in a later paper, further discussion of the point will not be entered into here.

#### SUMMARY

1. Hemp possesses a distinct vegetative and sexual dimorphism, but either sex may, under certain conditions, produce flowers of the opposite sex. The vegetative differences are the same for the plants which remain sexually pure and those which do not.

2. The ultimate height attained by hemp appears to be due largely to the length of the period of rapid rate of growth. This period of rapid rate of growth seems to be roughly proportional to the length of daily exposure to light. In a long daily light exposure the high growth rate extends over a long period and the plants are tall at maturity, but in a short daily exposure to light the period

of high growth rate is correspondingly shorter and the plants attain a height of only 1 to 2 feet. The growth rate of hemp grown in a short daily exposure to light may, however, at some time in its life exceed the growth rate of hemp grown in longer daily exposures.

3. The time of flowering of hemp is largely controlled by the relative length of day and night. A daily exposure to light of 7 hours seems to produce the greatest acceleration of the flowering process. A longer or a shorter daily exposure produces a retarding effect on flower development.

4. Three hours of light daily is the least in which hemp will grow for any length of time. If a shorter period of daily illumination is used the plants die before reaching the flowering stage.

5. It is not possible to retard the length of daily period of illumination sufficiently to prevent flowering without causing the death of the plants.

6. "Sex reversal" does not necessarily result when hemp is grown in the greenhouse during the winter months. More intersex types are found under such conditions, but many of the plants remain sexually pure. We may conclude that environment in some way affects the development of sex in this species, but the evidence shows that it does not control it.

7. Removal of flowers does not necessarily result in sexual modifications in hemp, although a few plants so treated and grown in a short daily exposure to light reversed their sex to the extent that some apparently pure staminate individuals matured seed. The occurrence of this phenomenon was not sufficiently general, however, to allow definite conclusions to be drawn. The removal of flowers from hemp which is grown in the field is without effect in causing sexual changes.

8. Although the development and expression of sex in hemp are affected by environmental factors, the changes produced are in many cases relatively minor ones, and a sweeping conclusion that genetic factors are in no way concerned with sex in this species is not warranted at the present time.

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### PLATE 1

**Right:** Staminate hemp plant grown in the greenhouse during the short days of winter. Note clustering of flowers and scarcity of leaves at the top.

**Left:** Carpellate hemp plant from seed planted at the same time as the plant on the right. The clustering of leaves at the tip and the inconspicuousness of the flowers are characteristic of the carpellate type.



# SPACING EXPERIMENTS WITH ACALA COTTON IN SOUTHERN CALIFORNIA<sup>1</sup>

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Different spacings of Upland cotton under irrigation in the Coachella Valley of California, which is located in Riverside County northwest of the Salton Sea, were compared in 1922 and 1923. Most of the cotton lands of the Coachella Valley are below sea level and the summer months are very hot. Though the extremely high temperatures prevailing during the summer months often cause excessive shedding of squares and young bolls, the season is so long that the plants are enabled to continue fruiting over a long period and large yields are usually obtained. Fields of Acala cotton planted as late as the tenth of June have sometimes yielded two bales to the acre.

In the Eastern cotton belt, where the crop season may be very short on account of dry weather, early frost, or boll-weevil damage, the ability of closer spaced plants to set a good crop in a short period is an important advantage. But in the irrigated valleys, where high temperatures interfere with the setting of an early crop, a long-continued fruiting period is necessary to obtain good yields. Under such conditions, it might be supposed that wide spacing, which permits the plants to grow large, would be an advantage. However, direct comparisons of spacings with the Acala variety of Upland cotton grown in the Coachella Valley indicate no advantage from wider spacing, even under the long-season conditions.

The usual practice in the valley is to chop the cotton out from 12 to 16 inches in the row. Spacing experiments were conducted in 1922 and 1923 to compare plants spaced at 12 and 6 inches in the row with "unthinned" cotton. As a rather heavy rate of seeding was used, thick stands were obtained and some plants had to be removed from the unthinned rows in order to prevent crowding where the stand was "bunchy." The plants were left 2 to 4 inches apart in the row, and this spacing has been designated as "2-inch" in the accompanying figures and tables. In thinning the 6 and 12 inch rows a measure was used and the plants were left as near these distances apart as the stand would permit.

The designated spacings indicate the distance between the majority of the plants rather than the average distance between plants, which is somewhat greater on account of occasional short skips in the stand. The plant counts contained in Table III, however, show the spacings to be very close to that designated, except in the case of the 2-inch spacing. The 6-inch spaced blocks contain about twice as many plants as the 12-inch blocks, and the 2-inch blocks contain about twice as many plants as the 6-inch blocks, so that the average spacing of blocks referred to as "2-inch" was about 3 inches, though much of the cotton was as close as 2 inches.

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<sup>1</sup> Received for publication May 16, 1924—issued November, 1924.

## SPACING EXPERIMENT IN 1922

The experimental material was planted May 17, 1922. The soil used was a fairly fertile sandy loam and quite uniform. The plants were thinned June 14 and 15, when they were 6 to 8 inches tall and had five to seven true leaves. To afford the best conditions for the 2-inch spacing it might have been better to reduce the stand in these rows when the plants were smaller, since that degree of crowding is sufficient to suppress the vegetative branches.

The experiment consisted of 24 rows 4 feet apart and 375 feet long. The 24 rows were grouped into five 4-row blocks with 2 guard rows on each side of the test to protect the outer blocks from "outside row effect." In blocks No. 1, 3, and 5 the plants were approximately 2 inches apart, while in block No. 2 the plants were spaced 6 inches apart and in block No. 4, 12 inches apart.

The plants were irrigated as would be good practice for a commercial field, without reference to the different spacings, as no difference in the water requirements of the different spacings was noticed. The plants grew to be from 4 to 5½ feet tall, the taller plants being on the east and west sides of the field.

The first picking was made October 10 and 11, which was somewhat too late to show differences in the earliness of the different spacings. The second picking was made December 20, about two weeks after the first frost.

In picking, the test was divided across the rows into three sections, thus making it possible to obtain six direct comparisons of the 6- and 12-inch spacing with the 2-inch spacing by comparing each section of the 6- and 12-inch spaced blocks with the section of 2-inch spacing on either side. These three sections are designated as A, B, and C, the length of rows in each section being 100, 125, and 150 feet respectively. The rows were not divided into sections of equal length because the plants on the east and west sides of the field were somewhat larger than those in the middle section, and the divisions were made so as to incorporate the larger plants in sections A and C and the smaller plants in section B.

The weight of the first and second pickings and the total yields from each section of each row are presented in Table I. The yields also are shown graphically in figure 1.

Six side-by-side comparisons of the 6 with the 2-inch spacing are possible by comparing the 6-inch plants in each of the three sections of block 2 with the 2-inch plants in the three sections of blocks 1 and 3, on either side.

In the first picking, made October 10, in five out of the six comparisons the 2-inch spacing outyielded the 6-inch, while in the second picking the 2-inch spacing led in only three of the six comparisons. In total yield, however, the 2-inch outyielded the 6-inch in five of the six comparisons.

Comparison of the total yields from each section of blocks No. 1, 2, and 3 shows that there was little difference in yield of the 6-inch and 2-inch spacings. The three sections of block No. 2 where plants were spaced 6 inches apart, yielded 63.0, 74.8, and 107.5 pounds, respectively, giving a total of 245.3 pounds for the entire block. The yields from each section of the two adjoining blocks of 2-inch spaced plants were as follows: Block No. 1, 64.2, 79.5, and 112.7 pounds, with a total of 256.4 pounds; block No. 3, 62.5, 75.6, and 110.8 pounds, with a total of 248.9 pounds. Thus in block No. 1 each section of 2-inch spaced plants outyielded the adjacent sections of 6-inch spacing, giving a total gain of 11.1 pounds for the entire block of 2-inch spaced plants. The 2-inch spacing in block No. 3 also outyielded the 6-inch spacing, but the gain was only 2.6 pounds.

Six comparisons are possible also between the 12- and 2-inch spacing, by comparing the 12-inch spaced plants in the three sections of block No. 4 with the

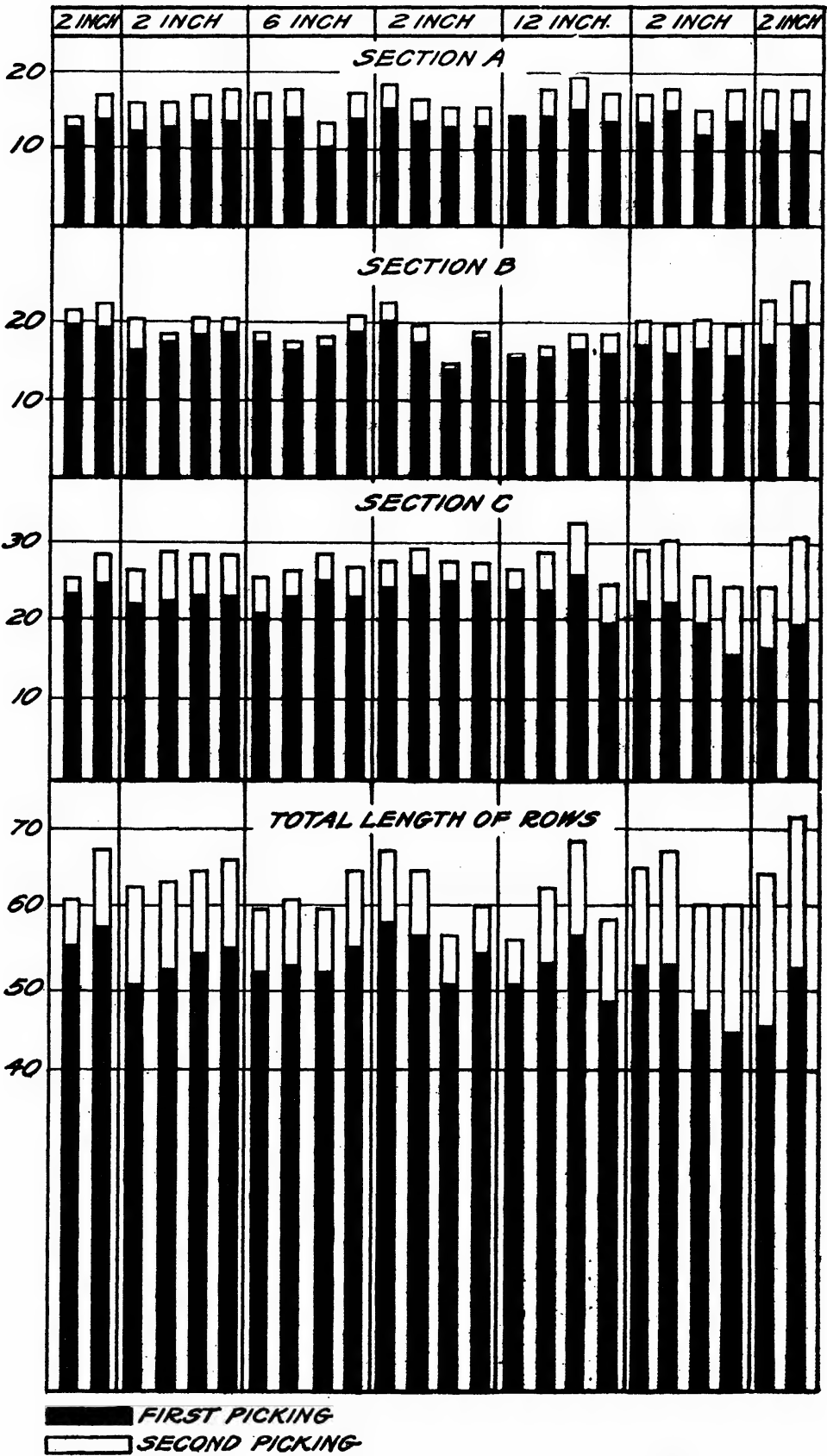


FIG 1.—Yield in pounds of the first and second picking by sections and total length of rows, spacing experiment, Coachella Valley, Calif., 1922

2-inch spaced plants in each of the three sections of blocks No. 3 and 5. The 2-inch led in half of the six comparisons of the first, second, and total pickings.

While the total yields recorded from blocks No. 3, 4, and 5 show that the 2-inch spaced plants outyielded the 12-inch spacing, the yields of the different sections of the 2-inch spaced blocks were not consistently higher than corresponding sections of 12-inch spacing. The three sections of block No. 4, where plants were spaced 12 inches, yielded 65.2, 69.5, and 111.2 pounds, respectively, giving a total of 245.9 pounds. In block No. 3, 2-inch spacing, the yields of sections A and C were less than those of the corresponding sections in block No. 4, and in block No. 5, 2-inch spacing, the yield of section C was less than that of the same section in block No. 4. But the total yields of blocks No. 3 and 5 were 248.9 and 253.0 pounds, respectively, the increase above the yield of 12-inch spacing being 3 pounds and 7.1 pounds, respectively.

Summarizing the total yields, the average yield of the three 2-inch blocks is found to be 252.8 pounds, while the yield of the 6-inch block is 246.3 pounds, and the 12-inch block 245.9 pounds, showing an increase of 3.2 per cent of 2-inch over 6-inch, and 2.8 per cent of 2-inch over 12-inch spacing.

However, these increases in the yield of 2-inch over 6- and 12-inch spaced plants, though rather consistent, are very small and an examination of Table I and figure 1 will show that there was considerable variation in the row yields. In order to compare the yields of the different spacings as a whole, the average yield of 25 feet of each section of each row was computed. This gave 12 yields in each block to calculate the mean yield of 25 feet of row length. These determinations follow in Table II.

The difference between the blocks in the average yield of 25 feet of row is so small that no significant difference can be shown between the different spacings. As there is no significant difference between the three 2-inch blocks they can be treated as one array, showing the mean yield of 25 feet of the 2-inch spacing to be  $4.1683 \pm 0.0485$  pounds, calculated on 36 cases, while the mean yield of 25 feet of 6- and 12-inch spacing are  $4.051 \pm 0.083$  and  $4.059 \pm 0.118$  pounds, respectively. Even with the lower probable error of the new mean of the 2-inch spacing no significant difference between any of the spacings can be found. Under the conditions of the experiments a difference of only 7.5 per cent would have been significant, but as the increase of 2- over 6-inch spaced plants was only 3.2 per cent and 2- over 12-inch spaced plants only 2.8 per cent, the different spacings must be regarded as yielding the same, and from a biometric standpoint there would be no assurance that the results would stand in the same order if the experiment were repeated under the same conditions.

The yields secured from this experiment show that plants spaced as close as 2 inches in the row yielded as well as the wider-spaced plants, indicating that no matter what the spacing the yields tend to be the same under the irrigated, long-season conditions of the Coachella Valley. It should also be noted that all blocks gave good yields, the highest, a 2-inch spaced block, yielding at the rate of 1,958.56 pounds of seed cotton or about 653 pounds of lint per acre.



TABLE I.—Yields of the first, second, and total pickings by sections of rows, total length of rows, and four-row block totals, cotton spacing experiment, Coachella Valley, Calif., 1922 <sup>a</sup>

Spacing	Row No.	Section A 100 feet			Section B 125 feet			Section C 150 feet			Total length of row		
		Yields			Yields			Yields			Yields		
		First picking	Second picking	Total pounds	First picking	Second picking	Total pounds	First picking	Second picking	Total pounds	First picking	Second picking	Total pounds
2-inch-----	Guard	{12.5 13.7	1.3 2.8	13.8 16.6	19.7 19.2	2.1 3.3	21.8 22.6	23.5 24.7	2.2 3.6	25.7 28.3	55.7 57.7	5.7 9.8	61.5 67.6
Block No. 1, 2-inch.	1-----	12.0	3.5	15.5	16.7	3.7	20.5	22.0	4.5	26.5	50.7	11.7	62.5
	2-----	12.7	3.0	15.7	17.0	1.6	18.6	22.5	6.5	29.0	52.2	11.1	63.3
	3-----	13.5	2.5	16.0	18.0	2.1	20.1	23.2	5.3	28.6	54.7	10.0	64.7
	4-----	13.5	3.5	17.0	18.5	1.8	20.3	23.0	5.6	28.6	55.0	11.0	66.0
	Total	51.7	12.5	64.2	70.2	9.2	79.5	90.7	21.9	112.7	212.6	43.8	256.5
Block No. 2, 6-inch.	1-----	13.2	3.0	16.2	17.5	.8	18.3	21.2	4.6	25.8	52.0	8.5	60.5
	2-----	13.7	3.6	17.3	16.2	1.2	17.5	23.0	3.1	26.1	53.0	8.0	61.0
	3-----	10.0	3.2	13.2	16.7	1.2	18.0	25.2	3.6	28.8	52.0	8.1	60.1
	4-----	13.7	2.6	16.3	18.7	2.2	21.0	22.5	4.3	26.8	55.0	9.2	64.2
	Total	50.6	12.4	63.0	69.1	5.4	74.8	91.9	15.6	107.5	212.0	33.8	245.8
Block No. 3, 2-inch.	1-----	14.5	3.0	17.5	20.2	2.3	22.6	24.0	3.3	27.3	58.7	8.7	67.5
	2-----	13.2	2.6	15.8	17.5	2.2	19.7	25.7	3.2	29.0	56.5	8.1	64.6
	3-----	12.5	2.2	14.7	14.0	.6	14.6	24.2	3.2	27.5	50.7	6.1	56.8
	4-----	12.5	2.0	14.5	18.0	.7	18.7	24.2	2.7	27.0	54.7	5.5	60.2
	Total	52.7	9.8	62.5	69.7	5.8	75.6	98.1	12.4	110.8	220.6	28.4	249.1
Block No. 4, 12-inch.	1-----	11.5	2.2	13.7	15.5	.5	16.0	23.5	2.7	26.2	50.5	5.5	56.0
	2-----	13.7	3.1	16.8	15.7	1.3	17.1	23.7	5.0	28.7	53.2	9.5	62.7
	3-----	14.5	3.7	18.2	16.5	1.8	18.3	25.5	6.6	32.1	56.5	12.2	68.7
	4-----	13.2	3.3	16.5	16.0	2.1	18.1	19.5	4.7	24.2	48.7	10.3	59.1
	Total	52.9	12.3	65.2	63.7	5.7	69.5	92.2	19.0	111.2	208.9	37.5	246.5
Block No. 5, 2-inch.	1-----	13.0	3.1	16.1	17.2	2.7	20.0	22.5	6.5	29.0	52.7	12.3	65.1
	2-----	14.5	2.7	17.2	16.0	3.8	19.8	22.0	8.0	30.0	52.5	14.6	67.1
	3-----	11.7	2.7	14.4	16.5	3.8	20.3	19.5	6.2	25.7	47.7	12.8	60.6
	4-----	13.2	3.5	16.7	15.7	4.0	19.7	15.7	8.3	24.1	44.7	15.8	60.6
	Total	52.4	12.0	64.4	65.4	14.3	79.8	79.7	29.0	108.8	197.6	55.5	253.1
2-inch-----	Guard	{12.0 13.2	4.7 3.0	16.7 16.2	17.2 19.5	5.8 5.5	23.1 25.0	16.2 19.5	8.0 10.8	24.2 30.3	45.5 52.2	18.6 19.3	64.1 71.6

<sup>a</sup> The weights were recorded to hundredths of pounds, but the yields are given in tenths of pounds. In computing the total yield hundredths of pounds were included if they equaled or exceeded a tenth of a pound; hence the total row yields in some instances are slightly greater than the sum of the three sections.

## SPACING EXPERIMENT IN 1923

In the season of 1923, another spacing experiment was conducted with Acala cotton, the same plant spacings being compared. The experimental material was planted on April 23, and located on approximately the same plot of land which was used in 1922.

The 1923 experiment had additional 6 and 12-inch blocks and consisted of 32 rows 3½ feet apart and 370.5 feet long. Acala cotton was planted on each side of the test, which protected the outer blocks from "outside row effects."

The plants were thinned between May 25 and June 5, the 2-inch spaced blocks being thinned first, when the plants were 8 to 10 inches tall and had about eight true leaves. Tomosis was very prevalent at the time of thinning and quite a number of plants having aborted terminal buds were pulled out in the thinning process where possible.

In the first four rows the plants were spaced 12 inches apart, the next four 2-inch, the next four 6-inch, and the next four 2-inch spacings were used, repeated once, making eight 4-row blocks in all. Thus blocks No. 1 and 5 were thinned to 12 inches apart, blocks No. 3 and 7 to 6 inches, and blocks No. 2, 4, 6, and 8 to 2 inches.

As in the 1922 test, this experiment was irrigated without reference to the different spacings. On one or two occasions, however, the whole test suffered somewhat for lack of water as the supply was not quite adequate during the hot summer months. The majority of the plants grew to be from 4½ to 5½ feet in height.

TABLE II.—Mean yield of 25 feet of row length in each block

Block No.	Spacing	Pounds
1.....	2-inch.....	4. 225±0. 071
2.....	6-inch.....	4. 051± .083
3.....	2-inch.....	4. 099± .101
4.....	12-inch.....	4. 059± .118
5.....	2-inch.....	4. 181± .067

Yields of the first, second, and total pickings are given by sections of rows, total length of rows, and 4-row block totals in Table III. Row yields of the first and second picking by sections and total length of rows are shown graphically in figure 2. The first picking was made September 21 to 24, which was early enough to show to some extent the relative earliness of the different spacings. The second picking was made December 20 and 21.

Nine direct comparisons are possible between the 2- and 12-inch spacings by comparing the yields of 12-inch spaced plants in each of the three sections of blocks No. 1 and 5 with the yields of 2-inch spaced plants in each of the 3 sections of blocks No. 2, 4, and 6 which are adjacent to the 12-inch blocks.

In the first picking the 2-inch spaced plants led in only five of the nine comparisons, but in the second picking the 2-inch spaced plants led in eight of the nine comparisons. In total yield the 2-inch also led in eight of the nine comparisons.

Twelve direct comparisons are possible between the 2- and 6-inch spacings by comparing the yields of 6-inch spaced plants in each of the three sections of blocks No. 3 and 7 with the yields of 2-inch spaced plants in each of the three sections of blocks No. 2, 4, 6, and 8, which are adjacent to the 6-inch blocks.

In the first picking the 2-inch spaced plants led in only five of the twelve comparisons and in one comparison the yields of adjacent 2- and 6-inch blocks were identical. In the second picking the 2-inch spaced plants led in ten, and in the total picking in nine of the twelve comparisons.

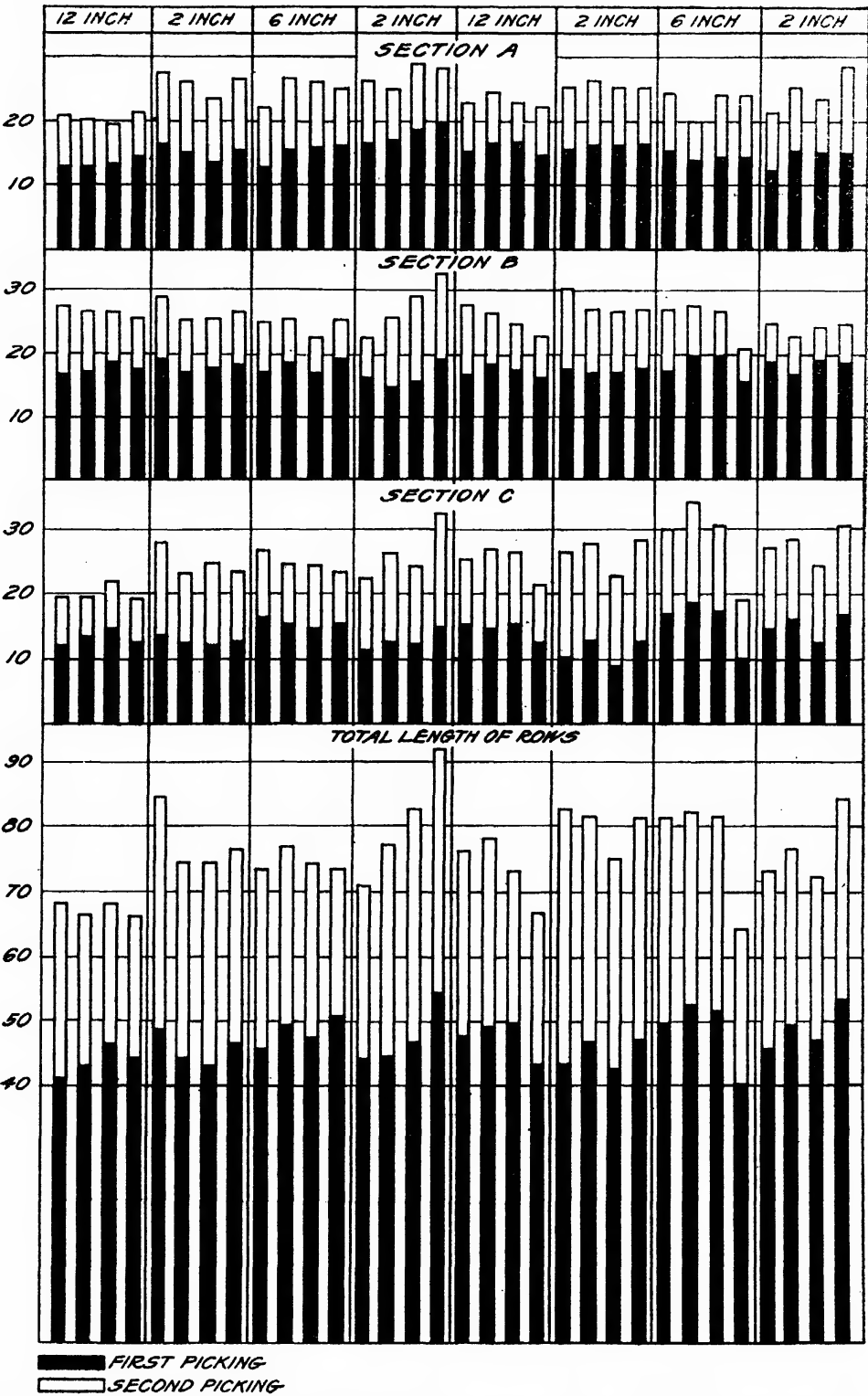


FIG. 2.—Yield in pounds of the first and second picking by sections and total length of cotton rows, spacing experiment, Coachella Valley, Calif., 1923

TABLE III.—Number of plants and yields of the first, second, and total picking by sections of rows, total length of rows, and four-row block totals, cotton spacing experiment, Coachella Valley, Calif., 1923

Spacing	Row No.	Section A				Section B				Section C				Total length of row			
		No. of plants	First picking	Second picking	Total pounds	No. of plants	First picking	Second picking	Total pounds	No. of plants	First picking	Second picking	Total pounds	No. of plants	First picking	Second picking	Total pounds
Block No. 1, 12-inch	1	111	12.7	8.5	21.2	120	16.5	11.2	27.7	108	12.0	7.5	19.5	339	41.2	27.2	68.4
	2	111	12.5	7.7	20.2	119	17.0	9.5	26.5	109	13.5	6.2	19.7	339	43.0	23.4	66.4
	3	107	13.2	6.5	19.7	119	18.5	8.0	26.5	118	14.5	7.5	22.0	344	46.2	22.0	68.2
	4	110	14.2	7.2	21.4	115	17.5	8.0	25.5	111	12.7	7.0	19.7	336	44.4	22.2	66.6
Total		439	52.6	29.9	82.5	473	69.5	36.7	106.2	446	52.7	28.2	80.9	1,358	174.8	94.8	269.6
Block No. 2, 2-inch	1	391	16.2	11.7	27.9	454	19.0	9.7	28.7	435	13.5	14.5	28.0	1,280	48.7	35.9	84.6
	2	354	15.2	11.0	26.2	363	16.7	8.5	25.2	333	12.2	11.2	23.4	1,050	44.1	30.7	74.8
	3	431	13.7	10.2	23.9	488	17.5	8.0	25.5	408	12.0	13.0	25.0	1,327	43.2	31.2	74.4
	4	455	15.5	11.2	26.7	443	18.0	8.2	26.2	395	12.7	11.0	23.7	1,293	46.2	30.4	76.6
Total		1,631	60.6	44.1	104.7	1,748	71.2	34.4	105.6	1,571	50.4	49.7	100.1	4,950	182.2	128.2	310.4
Block No. 3, 6-inch	1	195	12.7	9.5	22.2	231	17.0	7.7	24.7	207	16.2	10.5	26.7	633	45.9	27.7	73.6
	2	200	15.7	11.2	26.9	201	18.2	7.0	25.2	185	15.5	9.2	24.7	586	49.4	27.4	76.8
	3	196	16.0	11.2	27.2	216	16.7	6.0	22.7	203	14.7	10.0	24.7	614	47.4	27.2	74.6
	4	206	16.2	9.0	25.2	201	19.0	6.0	25.0	193	15.5	8.2	23.7	600	50.7	23.2	73.9
Total		797	60.6	40.9	101.5	849	70.9	26.7	97.6	787	61.9	37.9	99.8	2,433	193.4	105.5	298.9
Block No. 4, 2-inch	1	412	16.7	9.7	26.4	448	16.0	6.5	22.5	400	11.5	11.0	22.5	1,260	44.2	27.2	71.4
	2	417	17.0	8.2	25.2	442	14.7	11.2	25.9	438	12.7	13.7	26.4	1,297	44.4	33.1	77.5
	3	391	18.7	10.5	29.2	460	15.5	13.5	29.0	435	12.5	12.5	24.7	1,286	46.4	36.5	82.9
	4	316	20.0	8.2	28.2	463	19.2	13.0	32.2	440	15.0	17.2	32.2	1,219	54.4	38.4	92.8
Total		1,536	72.4	36.6	109.0	1,813	65.4	44.2	109.6	1,713	51.4	54.4	105.8	5,062	189.4	135.2	324.6
Block No. 5, 12-inch	1	91	15.7	7.5	23.2	116	16.5	11.2	27.7	107	15.7	9.7	25.4	311	47.9	28.4	76.3
	2	107	16.7	8.2	24.9	117	18.0	8.0	26.0	104	14.5	12.7	27.2	331	49.2	28.9	78.1
	3	103	17.0	6.0	23.0	112	17.2	7.0	24.2	115	15.7	10.7	26.4	330	49.9	23.7	73.6
	4	106	14.7	8.0	22.7	114	16.0	6.5	22.5	113	12.7	9.2	21.9	333	43.4	23.7	67.1
Total		407	64.1	29.7	93.8	459	67.7	32.7	100.4	439	58.6	42.3	100.9	1,305	190.4	104.7	295.1

Block No. 6, 2-inch	1	389	15.7	10.0	25.7	458	17.5	12.5	30.0	500	10.2	16.7	26.9	1,347	43.4	34.2	82.6
	2	398	16.7	10.0	26.7	441	16.7	10.0	26.7	410	13.0	15.0	28.0	1,240	46.4	35.0	81.4
	3	386	16.2	9.2	25.4	443	17.0	9.5	26.5	411	9.0	14.2	23.2	1,240	42.2	32.9	75.1
	4	383	16.7	9.2	25.9	411	17.7	9.2	26.9	399	12.7	16.0	28.7	1,193	47.1	34.4	81.5
	Total	1,556	65.3	38.4	103.7	1,753	68.9	41.2	110.1	1,720	44.9	61.9	106.8	5,029	179.1	141.5	320.6
Block No. 7, 6-inch	1	194	15.7	9.0	24.7	209	17.2	9.7	28.9	200	17.0	13.0	30.0	603	49.9	31.7	81.6
	2	216	14.0	6.2	20.2	216	19.5	8.0	27.5	200	18.7	15.7	34.4	632	52.2	29.9	82.1
	3	214	14.7	9.7	24.4	215	19.5	6.7	26.2	203	17.7	13.2	30.9	632	51.9	29.6	81.5
	4	222	14.5	9.7	24.2	211	15.5	5.0	20.5	199	10.0	9.7	19.7	632	40.0	24.4	64.4
	Total	846	58.9	34.6	93.5	851	71.7	29.4	101.1	802	63.4	51.6	115.0	2,499	194.0	115.6	309.6
Block No. 8, 2-inch	1	378	12.0	9.5	21.5	447	18.7	6.0	24.7	428	15.0	12.5	27.5	1,253	45.7	28.0	73.7
	2	448	15.5	10.0	25.5	372	17.7	5.0	22.7	382	16.2	12.5	28.7	1,202	49.4	27.5	76.9
	3	389	15.2	8.5	23.7	441	19.0	5.0	24.0	401	12.7	12.0	24.7	1,231	46.9	25.5	72.4
	4	378	18.2	10.7	28.9	342	18.5	6.0	24.5	403	17.2	13.5	30.7	1,123	53.9	30.2	84.1
	Total	1,593	60.9	38.7	99.6	1,602	73.9	22.0	95.9	1,614	61.1	50.5	111.6	4,809	195.9	111.2	307.1

The comparisons show that in the first picking the different spacings gave about the same yield, and that 2-inch spaced plants outstripped the wider spaced plants in the second picking, which gave the 2-inch spaced plants a greater total yield.

Comparisons of the total block yields show that the two blocks of 12-inch spaced plants were outyielded by three adjacent blocks of 2-inch spaced plants 15.1, 9.9, and 8.6 per cent, respectively. The increase of 2-inch over 6-inch spaced blocks was not so great. The two blocks of 6-inch spaced plants were outyielded by three adjacent blocks of 2-inch spaced plants 3.8, 8.5, and 3.5 per cent, respectively, but in one instance (block No. 8) an adjacent block of 2-inch spacing gave a decrease of 0.8 per cent. However, the mean yield of the four 2-inch spaced blocks is 315.67 pounds, of the two 6-inch blocks 304.25 pounds, and of the two 12-inch blocks 282.35 pounds, showing an increase of 3.7 per cent of 2-inch over 6-inch spaced plants, 11.8 per cent of 2-inch over 12-inch spaced plants, and an increase of 6-inch over 12-inch spaced plants of 7.8 per cent.

All spacings, however, gave good yields, the average production of the 2-inch spacing being at the rate of 2,651 pounds of seed cotton, or about 883 pounds of lint, which is a little over a bale and three-quarters per acre.

There are four rows 123.5 feet long in each of the three sections of each block, and mean yields of the first, second, and total picking of the twelve 123.5-foot rows in each block are given in Table IV.

TABLE IV.—Mean yield of twelve 123.5-foot row sections of blocks of 2-, 6-, and 12-inch spaced plants, Coachella Valley, Calif., 1923

Block No.	Spacing	First picking	Second picking	Total yield
		<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>
1	12-inch.....	14.56±0.42	7.90±0.16	22.48±0.59
2	2-inch.....	15.18±.44	10.68±.36	25.87±.33
3	6-inch.....	16.12±.30	8.79±.34	24.91±.29
4	2-inch.....	15.77±.55	11.27±.55	27.03±.60
5	12-inch.....	15.87±.26	8.72±.38	24.59±.37
6	2-inch.....	14.93±.55	11.79±.54	26.72±.32
7	6-inch.....	16.17±.51	9.63±.58	25.80±.84
8	2-inch.....	16.33±.43	9.27±.58	25.59±.52

In the first picking there is no significant difference between the two blocks of 12-inch spaced plants and any of the three adjacent blocks of 2-inch spaced plants, which was also the case with the two 6-inch spaced blocks in comparison with the four adjacent 2-inch spaced blocks.

Neither is there any significant difference in the yield of the first picking between the four 2-inch blocks, between the two 6-inch blocks, or between the two 12-inch blocks, though the difference in the latter instance is  $1.31 \pm 0.494$  which is almost three times the probable error.

Since there is no significant difference between blocks of like spacing each spacing can be treated as one array, but even with the reduced probable errors secured in this way there is no significant difference between any of the spacings in the yield of the first picking.

In the second picking 2-inch blocks showed increases in yield over adjacent 12-inch blocks of  $2.78 \pm 0.394$ ,  $2.55 \pm 0.667$ , and  $3.07 \pm 0.66$  pounds, all of which are considered significant differences, being 7.06, 3.83, and 4.65 times the probable error respectively.

The increase in yield of 2-inch spaced blocks over adjacent 6-inch blocks of the second picking was not so great as the increase over 12-inch spaced blocks. The differences in favor of the 2-inch are  $1.90 \pm 0.495$ ,  $2.49 \pm 0.647$ , and  $2.16 \pm 0.792$  pounds. The first two differences are to be considered significant as they are 3.84 and 3.85 times the probable error, but the last one in only 2.73 times

the error. In one comparison the 2-inch spaced block gave 0.36 of a pound less than the adjacent 6-inch spaced block, but this reduction is less than the probable error of either of the compared weights. However, as there is no significant difference between any of the blocks of like spacing each spacing can be treated as one array which gives an increase of 2-inch over 12-inch spaced plants of  $2.44 \pm 0.355$  pounds, which is 6.88 times the error, and an increase over 6-inch spaced plants of  $1.54 \pm 0.344$  pounds, which is 4.48 times the error. On this basis the writer finds that 2-inch spaced plants gave a significantly higher yield than 6- or 12-inch spaced plants in the second picking.

The total yields of the 2-inch spaced blocks gave an increase over adjacent 12-inch spaced blocks in every comparison. The increases are  $3.39 \pm 0.675$ ,  $2.44 \pm 0.70$ ,  $2.13 \pm 0.489$ , which are 5.02, 3.47, and 4.36 times the error and can be considered significant.

Although 2-inch spaced blocks outyielded adjacent 6-inch blocks in all but one case, where the difference was only 0.21 of a pound, these differences can not be considered significant.

Since there is no significant difference in total yield between any of the blocks of like spacing each spacing can be treated as one array, as with the first and second picking. On this basis the 2-inch spaced plants show an increase of  $2.77 \pm 0.441$  pounds over 12-inch spaced plants, which is 6.28 times the error. The increase of 2- over 6-inch spaced blocks, however, is only  $0.95 \pm 0.5$ , which should not be considered significant.

The mean yield of the first, second, and total pickings of 123.5 feet of row space is shown for each spacing in Table V.

TABLE V.—Mean yields of the first, second, and total pickings of 123.5 feet of 2, 6, and 12-inch spaced plants, Coachella Valley, 1923

Spacing	First picking	Second picking	Total yield
	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>
2-inch .....	15.55 $\pm$ 0.25	10.75 $\pm$ 0.27	26.30 $\pm$ 0.24
6-inch .....	16.14 $\pm$ .29	9.21 $\pm$ .34	25.35 $\pm$ .45
12-inch .....	15.22 $\pm$ .26	8.31 $\pm$ .23	23.53 $\pm$ .37

The results of the 1923 test not only substantiate the conclusion drawn from the 1922 test, that no reduction in yield occurs when plants are left as close together as 2 inches in the row, but indicate that increased yields may be secured from close-spaced plants. Although the first picking of the 1923 test showed no increase in the yields of the 2-inch spacings over the 6-inch and 12-inch, the 2-inch spacings forged ahead in the second picking, and gave significant increases in yield.

With plants spaced as close as 2 inches in the row there is a tendency to suppression or restriction of the development of the lower fruiting branches, but even in the first picking it is shown that this handicap is made up by the larger numbers of plants, so that the yields were not reduced below that of 6- or 12-inch spaced plants. The same degree of crowding does not continue throughout the season, as weaker plants are overtopped by their more vigorous neighbors, and as they become larger the plants spread apart, so the rows appear wider and the foliage less dense, allowing a full crop of bolls to be set and matured.

Figure 3 shows the block yields of the first and second picking of the different spacings for both the 1922 and 1923 experiments. It can readily be seen that the yields were about the same in the 1922 test, but the 1923 test shows a rather consistent alteration in block yields, the higher yields being coincident with the 2-inch spacing.

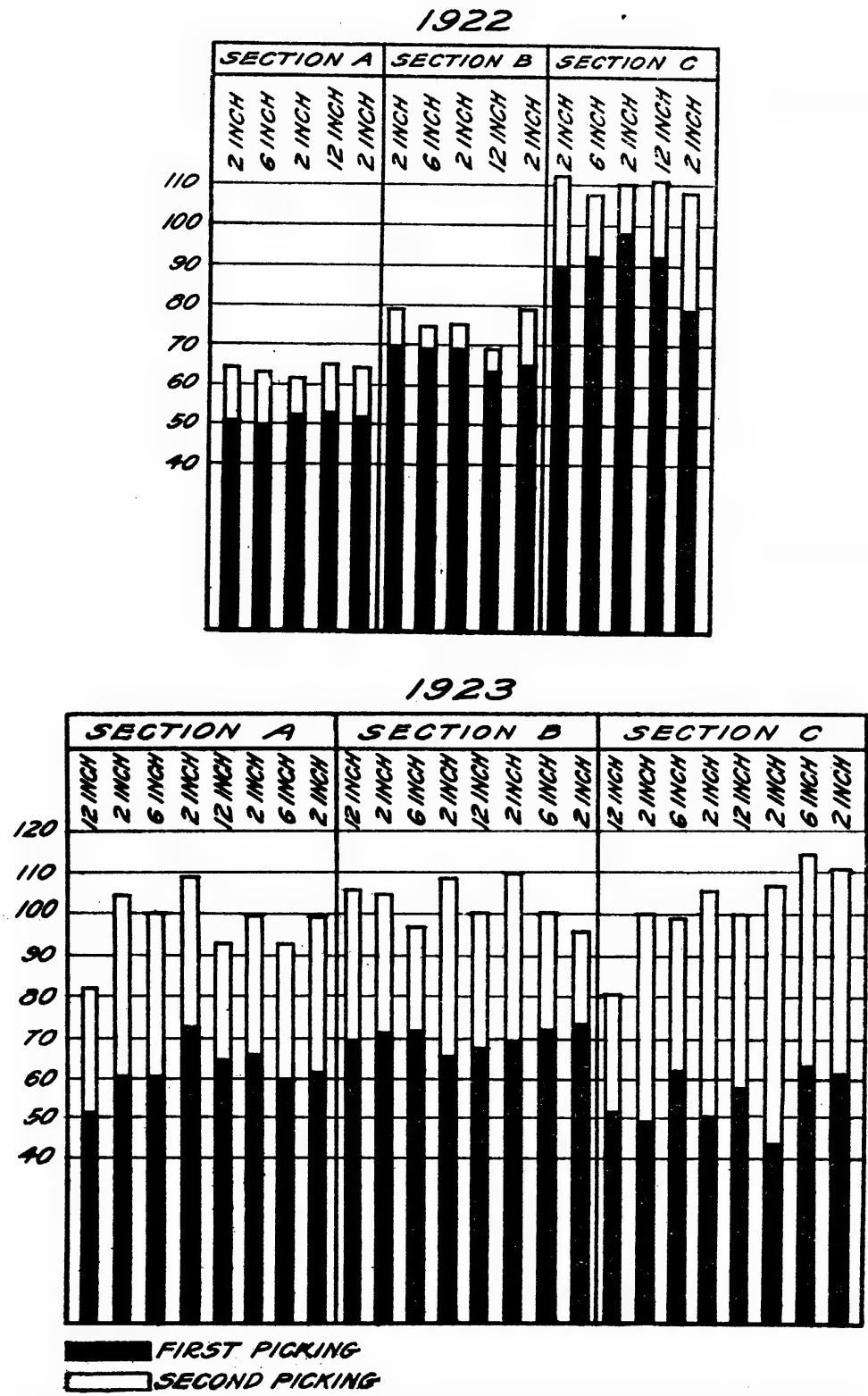


FIG. 3.—Block totals in pounds of spacing experiments, Coachella Valley, Calif., 1922 and 1923



Comparisons were not made between close spacings and large, rank, overgrown plants with many vegetative branches, which would be expected to show a greater advantage for close spacing, so that the present comparison between no thinning or 2-inch spacing with spacings of 6 and 12 inches is believed to be more significant. The 6- and 12-inch spaced plants were not crowded and did not have large numbers of vegetative branches, but an earlier thinning of these blocks would have allowed more vegetative branches to develop, with the result of greater crowding and closing of the lanes between the rows, which undoubtedly tends to reduce the yields and would have given the 2-inch spacing an additional advantage.

While large differences in yield between unthinned and wider-spaced plants in the irrigated valleys of the Southwest are not to be expected under conditions where both spacings are well grown, the ability of the close-spaced plants to set a good crop in a short time may often secure an advantage where the soil is not very well adapted to cotton culture, where the farmer is not familiar with the crop, or where irrigation is inadequate. Even if the greatest care is used in irrigation it is not to be expected that the plants can be kept in the best condition for the production of fruit throughout the entire season. Water is likely to be held off too long occasionally, which causes shedding, or may be applied too frequently, causing overgrowth and consequent shedding. The degree of this kind of damage is in proportion to the skill with which the farmer irrigates his cotton. On this basis it becomes apparent that the ability of the close-spaced plants to set more bolls during short periods of favorable conditions may also be of value in irrigated districts. This does not mean that good yields are not secured from the wide-spaced plants under favorable conditions, but that the chances of a full crop, especially under less favorable conditions apparently are better with close spacings than with wide spacings, even under the long-season conditions of the irrigated valleys.

### CONCLUSIONS

Experiments under irrigation in the Coachella Valley of California of plants spaced approximately 2, 6, and 12 inches apart in the row indicate that no reduction in yield occurs when plants are left as close as 2 inches in the row, and that occasionally increased yields may be obtained from 2-inch spaced plants, even under long-season, weevil-free conditions.

It also is indicated that plants spaced at 6 inches give equal or better yields than 12-inch spaced plants. However, since the 6-inch spacing is not convenient in field operations the practical alternatives are 2 plants together at 12 inches or omitting the chopping operation entirely, except in thick stands, when some plants should be pulled out.

In the first picking the yields were nearly the same for all spacings; in the second picking the 6 and 2-inch spacings yielded more than the 12-inch, the greatest increase being in the 2-inch spacing.

Though unthinned cotton, or plants spaced as close as 2 inches in the row, is more crowded at first, which may result in a suppression of some of the lower fruiting branches, as well as of the vegetative branches, the crowding is lessened by the spreading apart of the plants as they become larger. This is in contrast with the behavior of plants that are widely spaced at first, which produce numerous vegetative branches and become more crowded as the season advances.

Since even on the best soil and with the greatest care it is not likely that the plants can be kept in the best condition for the production and retention of fruit throughout the entire season, the ability of the close-spaced plants to set a crop rapidly during favorable periods is likely to prove of advantage even under the long-season, weevil-free conditions of the Southwest.



# CORRELATION AMONG QUANTITATIVE CHARACTERS IN MAIZE<sup>1</sup>

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Varieties of maize differ greatly in general size and in the length of time required to produce a crop. As a rule, the large varieties require a longer growing period and produce a greater yield than the small varieties.

The breeder, in producing a type of maize for special conditions, often is confronted with the task of combining the characters of high productivity with earliness and therefore it is desirable to know to what extent the various characters of size and season are correlated among themselves.

If intervarietal correlations were calculated it would be found that the characters of size and season were very closely associated, but such correlations would afford little basis for predicting the facility with which such combinations could be dissolved through hybridization. With many combinations there are, of course, physiological limitations which can not be surmounted, but a consideration of the varieties in existence shows that physiological limitations permit a wide range of combinations.

It has been shown that size characters in maize are inherited and that the mode of inheritance can be explained in accordance with Mendelian principles by hypothecating numerous stable heritable elements or factors.<sup>2</sup> Since these factors are distributed among 10 chromosomes it follows that numerous genetic correlations among size characters are to be expected, but while such correlations increase the difficulty of making new combinations they seldom are insurmountable.

Correlations among various characters of maize have been studied by several investigators, chiefly in searching for a criterion of selection within a variety.<sup>3, 4, 5</sup> Such studies have shown an almost complete independence of the characters of the ear with subsequent yield or other size characters, but since they were made within a single variety in each case they do not afford a secure basis for predicting complete freedom of recombination in hybrids between diverse forms.

## MATERIAL

The present investigation was undertaken with a view to measure the correlations among certain quantitative characters in hybrids between varieties of maize offering the maximum size differences.

As the diminutive parent the writer has used the well-known Tom Thumb variety of pop corn which, excluding abnormal dwarf forms, probably is the

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<sup>1</sup> Received for publication June 27, 1924—issued November, 1924.

<sup>2</sup> EMERSON, R. A., and EAST, E. M. THE INHERITANCE OF QUANTITATIVE CHARACTERS IN MAIZE. Nebr. Agr. Exp. Sta. Research Bul. 2, 120 p., illus. 1913.

<sup>3</sup> EWING, E. C. CORRELATION OF CHARACTERS IN CORN. N. Y. Cornell Agr. Exp. Sta. Bul. 287, p. 67-100, illus. 1910.

<sup>4</sup> LOVE, H. H. THE RELATION OF CERTAIN EAR CHARACTERS TO YIELD IN CORN. Ann. Rpt. Amer. Breeders' Assoc. 7 : 29-40. 1912.

<sup>5</sup> LOVE H. H., and WENTZ, J. B. CORRELATION BETWEEN EAR CHARACTERS AND YIELD IN CORN. Jour. Amer. Soc. Agron. 9 : 315-322. 1917.

smallest type of normal maize. The gigantic parent used is a variety of maize from the west coast of Mexico, designated Jala.<sup>6</sup> Ears and seeds of both varieties

are shown in Plate 1 and the relative differences in size between them are shown diagrammatically in figure 1.

The cross was made with the large variety as the female parent and a small  $F_1$  population was grown at Lanham, Md. From this  $F_1$  population two self-pollinated ears were chosen to provide the  $F_2$  generation. One of these ears was perfectly normal with yellow, light yellow, and white seeds in the proportion of 1-2-1 (100 yellow, 246 light yellow, 119 white) while the other had but two seed classes, yellow and white in approximately equal numbers (170 white, 163 yellow) with a large proportion of deficient seeds consisting of hardly more than a pericarp. Since the Tom Thumb parent has a yellow endosperm and that of the Jala parent is white it was thought possible that the large proportion of deficient seeds represented some incompatibilities in recombinations.

These  $F_2$  populations together with progenies of both parents and the remainder of the  $F_1$  seed were grown at Chula Vista, Calif., where the moderate climate and long growing season permitted the latest segregates and parents to mature normally.

In view of the absence of light yellow seeds on one ear, together with the high proportion of sterile ovularies, the several seed classes were grown separately. This proved to be a needless precaution, as there were no outstanding differences between the two  $F_2$  populations nor in the several seed classes, as is shown by the biometrical constants in Table I. In analyzing the correlations, however, only one  $F_2$  population was used, namely, that not having the complication of deficient seeds. The correlations were calculated separately for each seed class but since there were no significant differences between the classes only the coefficients for the entire population are presented.

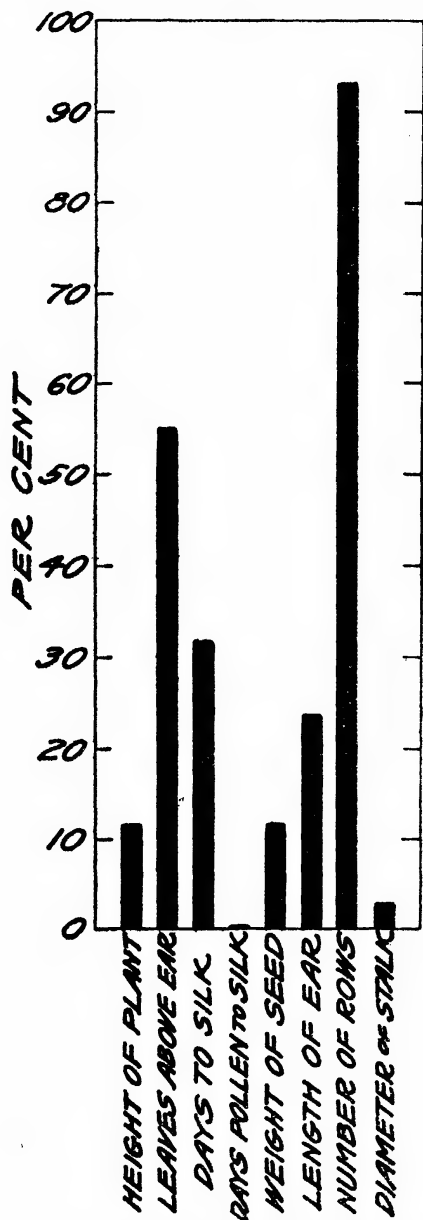


FIG. 1.—Diagrammatic representation of the differences between Jala and Tom Thumb maize. Jala taken as 100

<sup>6</sup> KEMPTON, J. H. JALA, A LARGE VARIETY OF CORN FROM MEXICO. Jour. Heredity 15 :337-344. 1924.

### INHERITANCE OF THE QUANTITATIVE CHARACTERS

The designations of the characters studied are self-explanatory, with the possible exception of "weight of seed," which is the average weight of a single seed produced by the plants measured, not the weight of the seed planted. The frequency distributions for several of the characters in which the difference between the parents was greatest are shown in figures 2 to 5. These polygons include in

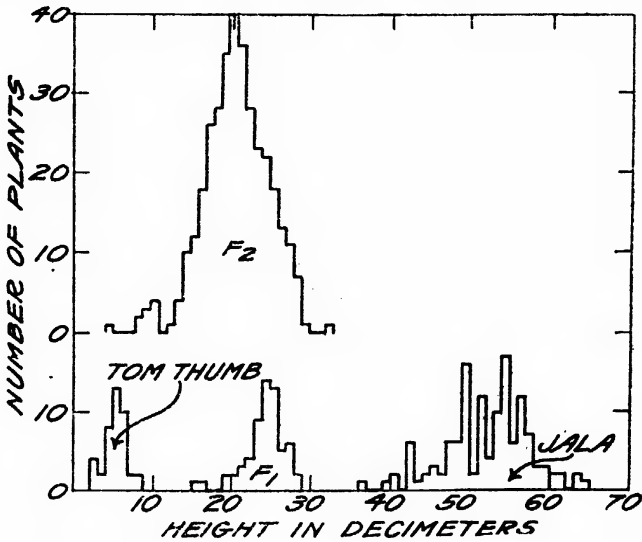


FIG. 2.—Frequency distribution for height of plant. Estimated factorial difference between parents 28

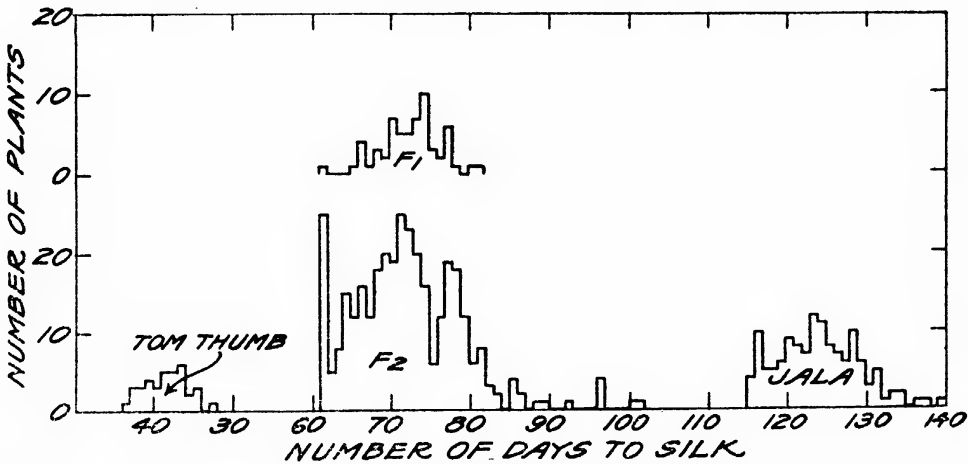


FIG. 3.—Frequency distributions for days to silk. Estimated factorial difference between parents 26

each case the parents, the  $F_1$  and the  $F_2$  generations. In all the characters studied except number of rows on the ear, the  $F_1$  generation may be said to be intermediate between the parents, though actually below the mean of the parents in five cases and above in both the length of ear and in number of rows. In some cases the size of the larger parent is approximated. With number of rows the  $F_1$  exceeds the larger parent, the increased number tending to be maintained in the  $F_2$  remaining above the mean of the parents.

TABLE I.—*Biometrical constants for Jala and Tom Thumb maize and their F<sub>1</sub> and F<sub>2</sub> hybrid populations*

Character designation	Con- stants	Jala	Tom Thumb	F <sub>1</sub>	F <sub>2</sub> with normal seed classes				F <sub>2</sub> with deficient seeds		
					White seeds plant- ed	Yellow seeds plant- ed	Pale yellow seeds plant- ed	Entire popu- lation	White seeds plant- ed	Yellow seeds plant- ed	Entire popu- lation
Height in dcm.	mean....	52.75	6.14	24.80	21.86	21.20	20.80	21.17	22.80	21.10	21.69
	σ.....	5.06	1.46	2.59	4.16	3.66	4.15	4.06	4.59	3.92	4.20
	PE.....	.30	.15	.222	.291	.320	.204	.147	.632	.542	.34
Number of leaves above ear.	mean....	5.98	3.32	4.41	3.98	3.85	3.94	3.94	4.50	4.48	4.49
	σ.....	.78	1.63	.53	.84	.87	.72	.76	.64	.91	.79
	PE.....	.05	.17	.045	.059	.076	.036	.028	.089	.091	.064
Number of days to silk.	Mean....	124.7	39.50	73.20	74.22	71.10	72.50	72.73	77.80	74.31	75.52
	σ.....	7.44	2.52	3.93	5.17	7.34	7.66	7.06	10.17	6.74	8.26
	PE.....	.44	.28	.342	.360	.639	.385	.26	1.39	.673	.67
Days pollen to silk.	Mean....	6.20	0.00	1.91	1.73	.88	1.56	1.49	1.50	2.04	1.86
	σ.....	2.88	1.89	1.91	2.26	1.33	2.55	2.28	1.68	3.21	2.78
	PE.....	.18	.23	.167	.160	.114	.128	.084	.231	.322	.226
Weight of a single seed in grams.	Mean....	.562	.064	.310	.28	.28	.27	.27	.25	.25	.25
	σ.....	.34	.02	.030	.05	.03	.04	.05	.07	.04	.05
	PE.....	.04	.003	.003	.004	.003	.002	.002	.01	.005	.004
Length of ear in cm.	Mean....	26.25	6.22	20.00	16.73	15.60	16.50	16.42	18.20	17.79	17.94
	σ.....	5.54	1.49	2.14	2.79	2.33	3.21	2.73	3.37	2.80	3.01
	PE.....	.30	.19	.185	.191	.230	.161	.100	.463	.29	.248
Number of rows on ear.	Mean....	12.68	11.80	14.05	13.19	13.40	13.13	13.19	13.20	12.80	12.93
	σ.....	1.74	.69	1.13	1.78	1.24	1.64	1.62	1.45	1.56	1.67
	PE.....	.10	.10	.097	.122	.108	.083	.060	.199	.16	.139
Diameter of stalk in mm.	Mean....	39.05	-----	28.16	26.92	26.60	27.07	26.95	27.00	25.26	25.88
	σ.....	4.27	-----	2.56	5.47	5.38	5.70	5.50	5.08	4.37	4.60
	PE.....	.25	-----	.22	.375	.469	.281	.200	.70	.44	.374

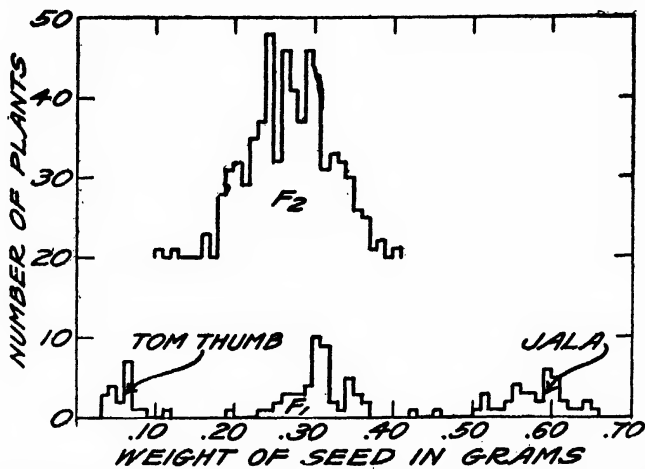


FIG. 4.—Frequency distributions for weight of seed. Estimated factorial difference between parents 19

By use of Castle's formula as improved by Wright <sup>7</sup> for estimating the factorial difference in the case of each character, it is seen that the least number of factors involved for the four characters, days to silk, height of plant, length of ear, and weight of seed, is with the character, length of ear, where the formula indicates a difference of 17-18 factors between the two parents, while 28 factors are indicated for height of plant.

While too much emphasis should not be placed upon this method of estimating the number of heritable factors involved, it is believed that the method underestimates rather than exaggerates the actual number. This would seem to follow

<sup>7</sup> CASTLE, W. E. AN IMPROVED METHOD OF ESTIMATING THE NUMBER OF GENETIC FACTORS CONCERNED IN CASES OF BLENDING INHERITANCE. Science 54: 223. 1921.

from the fact that in maize the occurrence of more than 20 factors almost certainly would necessitate linkages. If the linkage were perfect the effect would be of a single factor and as the degree of linkage decreased the effect of two independent factors would be approached.

With the exception of the number of days to silk, the  $F_2$  distributions are fairly regular and very similar to those for the plants of the  $F_1$ . The distribution for number of days to silk suggests trimodality with a large group of early plants and a fairly well-defined population of late plants. However, the parental ranges are not reached and no plants were obtained as early as the latest of the early parent nor as late as the earliest plants of the late parent and the factorial difference between the parents is estimated at 26.

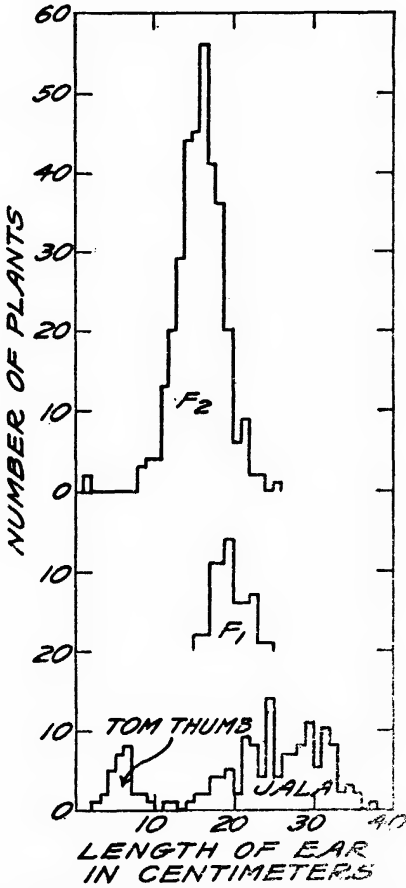


FIG. 5.—Frequency distributions for length of ear. Estimated factorial difference between parents 17-18

In the other characters the parental types were represented in the extreme plants of the  $F_2$  distributions, but the parental ranges were not exceeded except in the previously-mentioned case of number of rows on the ear. No frequency polygon is given for this latter character, since the parental difference was not large.

### CORRELATION BETWEEN THE CHARACTERS

The coefficients of correlation among the characters of the  $F_1$  plants may be taken as an indication of the physiological as distinct from the genetic relationships. An examination of the  $F_1$  coefficients given in Table II shows that there is only one significant correlation, namely, that between length of ear and number of rows, indicating that the conditions which favored the production of long ears also resulted in the development of a large number of rows.

TABLE II.—*Coefficients of correlation among the characters of the plants of the F<sub>1</sub> of Jala × Tom Thumb<sup>a</sup>*

Character designation	Height	Leaves above ear	Days to silk	Pollen to silk	Weight of seed	Length of ear	Number of rows	Diameter of stalk
Height.....		0.26	0.23	0.18	0.12	0.28	0.16	0.14
Leaves above ear.....	0.26		.13	.05	-.07	.16	.22	.00
Days to silk.....	.23	.13		.02	-.23	.07	.12	-.05
Pollen to silk.....	.18	.05	.02		.10	.05	.26	-.00
Weight of seed.....	.12	-.07	-.23	.10		.37	.11	.03
Length of ear.....	.28	.16	.07	.05	.37		.43	.11
Number of rows.....	.16	.22	.12	.26	.11	.43		.07
Diameter of stalk.....	.14	.00	-.05	-.00	.03	.11	.07	

<sup>a</sup> Coefficients in excess of 0.272 are greater than 3 times their error.

From these F<sub>1</sub> coefficients the conclusion seems justified that physiological correlations are not important within the ranges of the characters studied, but the ranges involved in the F<sub>1</sub> are in no case extreme.

Proceeding to the correlations among the characters of the second generation, it will be seen from an examination of Table III that the closest correlations are with height of plant. Curiously enough, the correlation of plant height with length of season, as measured by the number of days to silk, is not high, though the plants of the second generation ranged from 4 to 33 decimeters in height and required from 62 to 102 days to silk.

The length of season or days to silk is also correlated with the weight of the seed and with the length of the ear, but since the height of the plant is rather closely correlated with ear length, it may be urged that the relationship between season and ear length is an indirect result of this correlation. Calculating the partial correlation for season and length of ear for constant height of plant, it is found that though the relationship between ear length and season is reduced to .131 the correlation remains significant. Treating the correlation between season and weight of seed in the same manner, the partial correlation for constant height becomes .096 or negligible.

TABLE III.—*Coefficients of correlations among the characters of the plants of the F<sub>2</sub> of Jala × Tom Thumb<sup>a</sup>*

Character designations	Height	Number of leaves above ear	Number of days to silk	Number of days from pollen to silk	Weight of seed	Length of ear	Number of rows	Diameter of stalk
Height.....		0.107	0.174	0.044	0.350	0.457	0.025	0.568
Number of leaves above ear.....	0.107		.089	.097	.062	.108	.162	.098
Number of days to silk.....	.174	.089		.338	.149	.194	-.093	.107
Number of days from pollen to silk.....	.044	.097	.338		.105	.071	-.027	.065
Weight of seed.....	.350	.062	.149	.105		.382	-.087	.290
Length of ear.....	.457	.108	.194	.071	.382		.084	.380
Number of rows.....	.025	.162	-.093	-.027	-.087	.084		.039
Diameter of stalk.....	.568	.098	.107	.065	.290	.380	.039	

<sup>a</sup> Coefficients in excess of 0.119 are greater than 3 times their error.

From a consideration of the correlations with days to silk there would seem to be no great obstacle to combining earliness with large ears, heavy seeds, or even tall plants. On the other hand, more difficulty would be encountered in attempting a combination of short plants with long ears and heavy seeds or in producing stocky plants by combining short stature with large diameter.



It seems worthy of note that while the plants of the  $F_1$  showed a close correlation of ear length with number of rows, this correlation shrinks essentially to zero among the plants of the second generation.

Contrary to the conditions found among some animals where the factors affecting size are found to be general for the organism, the lack of correlations among several of the characters studied in this hybrid would seem to indicate a rather high degree of independence of the factors influencing the various parts of the plants.

When the probable number of heritable factors involved in these size characters is taken into consideration, together with the low degree of correlation, it seems certain that the distribution of these factors is fairly general among the 10 chromosomes, lending support to the view that in maize the chromosomes are more or less duplicates of each other, each having complementary factors for most of the characters.

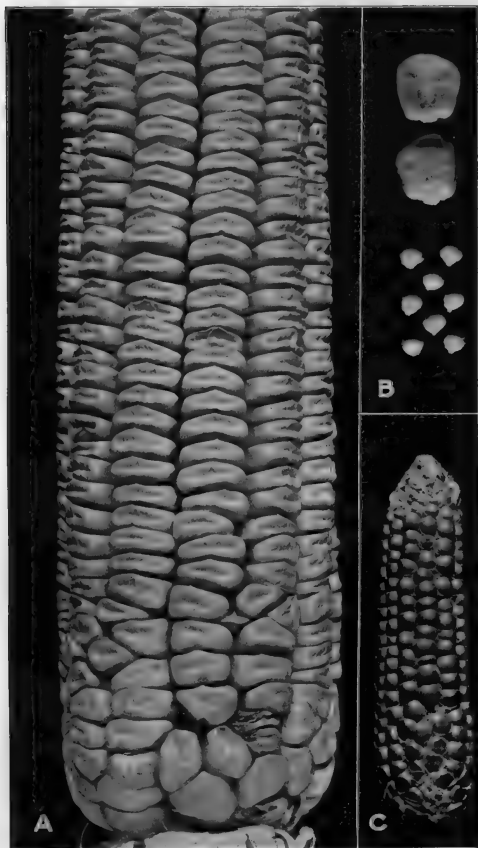
### SUMMARY AND CONCLUSION

The correlations among certain quantitative characters of maize have been studied in a hybrid between the smallest and one of the largest varieties. While apparently there are a great many heritable factors involved in the several differences between large and small varieties of maize, the correlations among the characters of size often are low or negligible and there seems to be no genetic obstacle to forming recombinations of desirable quantitative characters by hybridization.

PLATE 1

Section of an ear of Jala maize, an ear of the Tom Thumb variety, and seeds of both showing differences in size. (Natural size.)

(1102)



# EFFECT OF FUMIGATION UPON HEATING OF GRAIN CAUSED BY INSECTS<sup>1</sup>

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The heating of grain in storage is one of the serious problems confronting the farmers and grain dealers. A moisture content in wheat of 14.5 per cent has been fixed by the Bureau of Agricultural Economics of this department as a line between safety and possible danger, so far as the moisture content affects the keeping quality of wheat in storage. It is well known that the heating of grain may be caused by a high moisture content, and grain dealers have been put to much expense in devising methods of quickly detecting unusual rises of temperature in stored grain (by means of electric thermometers, for instance), and in preventing its continuance by transferring from bin to bin, by mixing lots of grain of different moisture content, or by passing the grain through drying machines. With heating due to causes other than insect infestation, this paper does not deal.

From the foregoing statements it may be assumed that when wheat shows a moisture content of less than 14.5 per cent, it is likely to remain in condition if given proper storage. Grain dealers, farmers, and seedsmen are aware, however, that seeds containing a lower percentage of moisture may heat badly if they become infested with insects. Thus, wheat arriving at the Baltimore grain terminals during 1923 was found heating as a result of infestation by the Angoumois grain moth<sup>2</sup> when its moisture content was but 12 per cent. In farmers' bins wheat is very often found heating as a result of attack by the rice<sup>3</sup> and granary weevils,<sup>4</sup> while beans, cowpeas, and chick-peas are commonly infested by bruchids, and heat badly as a result.

During the season of 1919-20, G. H. Baston, of the then Bureau of Markets and Crop Estimates of this department, recorded (15)<sup>5</sup> observations made during the storage at Buffalo, N. Y., of 15,000,000 bushels of wheat belonging to the war-time Grain Corporation. In spite of the fact that this wheat had an average moisture content ranging from 11.3 to 12.6 per cent, it was found during the winter months that it was in part going out of condition, and it became necessary to run the bins to determine the actual state of the grain. Nine hundred and seventy-seven bins were transferred and examinations made; of these, 374 were in good condition, 396 were "found to contain a trace of weevil and beetle infestation,"<sup>6</sup> and 207 were found "infested with weevil and beetle." By taking

<sup>1</sup> Received for publication Apr. 22, 1924—issued November, 1924.

<sup>2</sup> *Sitotroga cerealella*.

<sup>3</sup> *Sitophilus oryza*.

<sup>4</sup> *Sitophilus granarius*.

<sup>5</sup> Reference is made by number (italic) to "Literature cited," p. 1116.

<sup>6</sup> By "trace of weevil and beetle" it is meant that specimens of insects were found among the kernels which were not apparently damaged, although they may have contained the immature stages; the term "weevils" refers primarily to the rice weevil, *Sitophilus oryza*, and to a lesser extent to the granary weevil, *Sitophilus granarius*; the term "beetle" refers to the flour beetle, *Tribolium confusum*, to the flat grain beetle, *Cryptolestes pusillus*, and to the saw-toothed grain beetle, *Oryzaephilus surinamensis*, and possibly to several others of the smaller grain beetles.

samples of grain for moisture analyses, and taking temperature readings at the time the grain was run from bin to bin, Mr. Baston gathered the data presented in Table I.

From these data he concludes that the temperature was highest in the bins which were infested, that the moisture content was lower in the bins which showed a trace of infestation or a pronounced infestation with weevil, and that these facts indicate that the moisture was not the factor that caused the rise in temperature. "Eliminating the factors of moisture, damage, and time in storage, one by one," the data indicate "plainly," so he states, "that the rising temperature was caused by the weevil and the beetle." These conclusions are shown diagrammatically in figure 1, the left-hand end of which represents the conditions of temperature and moisture for the uninfested grain, the right-hand end those for the highly infested grain, and the center those for that with a trace of infestation.

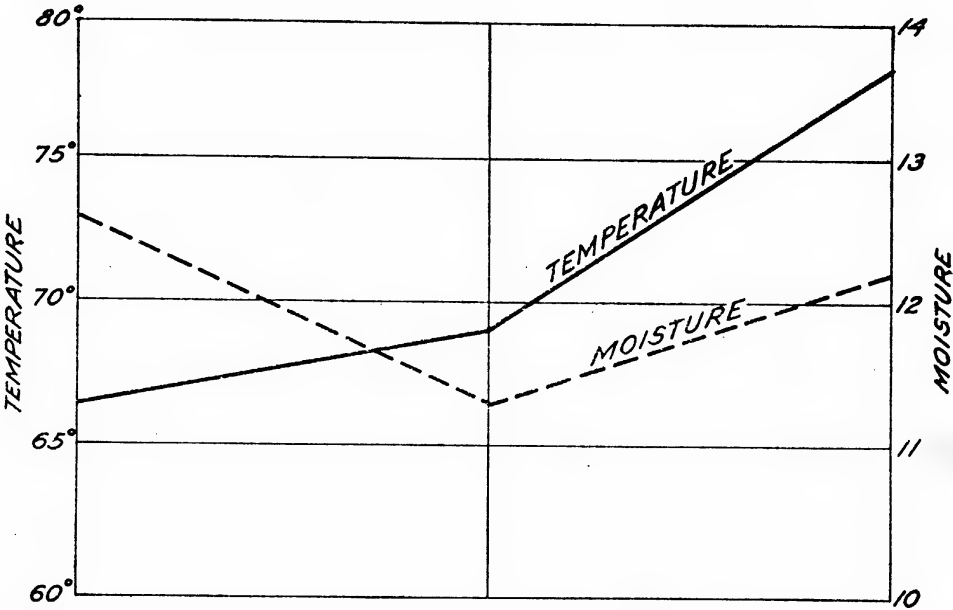


FIG. 1.—Diagram showing average condition of 977 bins of wheat handled at Buffalo, N. Y., in 1919-20

TABLE I.—Correlation of general condition of wheat in 977 bins, showing average moisture, average temperature, and other data, for good, slightly infested, and highly infested wheat

General condition	Average moisture content	Damaged wheat (average)	Time in storage	Number of bins of wheat	Percentage of bins of wheat	Average temperature
		Per cent	Days			° F.
Good. Free of insects.....	12.6	3.1	105	374	38.3	66.2
Showing trace of insects.....	11.3	2.9	118	396	40.5	69.3
Highly infested.....	12.2	2.9	90	207	21.2	78.3

With these conclusions, entomologists who have had experience with seed-infesting insects can readily agree. Considering the numerous instances of heating seeds with which the writers have come in contact during the past few years, it seems strange that in 1892 Doran should state (6, 7) that he knew of

but three previously published records in American entomological literature containing reference to high temperatures caused by grain-infesting insects.

Dr. L. O. Howard, in 1888, records (3) the sending to the then Division of Entomology by Judge Lawrence Johnson at Holly Springs, Miss., of seeds of *Dolichos* species badly infested with *Bruchus scutellaris*. These seeds had been stored in a paper sack of a gallon capacity, filling it about one-third full. With the temperature of the air surrounding the bag standing at 71° F., a thermometer placed in the seeds recorded 96° F. The difference of 25° F. was thought to be due in great part to a mechanical cause—the gnawing of the peas by the beetles and their larvae.

W. D. Richardson, of Frederick, Va., in a letter of November 1, 1891, addressed to Dr. E. A. Schwarz and published in "Insect Life" (5), states that on August 13, he had occasion to move a bag of peas which had stood in a corner of a room in his house for a month or more, and from which thousands of *Bruchus scutellaris* had been emerging. On touching the bag he was surprised at the temperature, and discovered that while the temperature of the room was 70° F., that within the sack of peas registered 88° F. This temperature continued for about two weeks, when it commenced to fall and the beetles soon thereafter ceased to emerge. Prof. A. J. Cook stated in 1889 (4) that insects had been known to produce heat in grain, but gave no temperature records.

Doran states (7) that in February, 1892, at the Maryland Agricultural College his attention was called to a bin of bran or middlings 12 feet long by 6 feet wide, which was badly infested with *Silvanus (Carthartus) cassiae*. He discovered that, with the room temperature at 53° F., the temperature of the bran taken about 1½ feet below the surface ranged from 42° to 95° F. These extremes of 42° and 95° F. were obtained from spots in the bin not more than 3 feet apart. The temperature of 95° F. was confined to a small space not more than 2 to 3 feet in diameter, and within this the beetles and their larvae were very numerous. He states that the bran was "perfectly dry with no indication of mold or ferment." The lower temperature of 42° F., or near that, was found in various places in the same bin and in the bins and sacks of bran stored in the same room. Doran took a sample of bran from the spot showing the highest temperature and found 1 pound of it to contain about 1,500 beetles. There were 103 weevils in an ounce of bran, which gave an estimate of 1,648 beetles to the pound. As 100 of these beetles weighed 23 grains, those in a pound of bran were estimated to weigh about 345 or 350 grains, or about one-twentieth of the entire weight of the bran and insects combined.

Lintner in 1893 wrote (8) that wheat in Montgomery County, Pa., in 1892, was so badly infested with the Angoumois grain moth that when sacked directly from the threshing machine it would heat overnight. In 1901, Dr. L. O. Howard stated (9) that this pest was so abundant in the 1900 crop in Pennsylvania, New Jersey, and Delaware wheat that the grain heated badly. Dr. F. H. Chittenden observed (10) that heating may be caused independently of the attack of insects, but an abundance of insects in stored cereals frequently causes such heating that the temperature of infested material may be increased 25° to 40° F. above the temperature of the surrounding air. In 1915 Newell in Texas wrote (12) of the heavy infestation of sudan grass seed by the Angoumois grain moth, the peculiarity of the infestation being the resulting heating of the infested seed. Back (16) in 1922 gave data on the heating of chick-peas infested with the four-spotted bean weevil. Heating of wheat as a result of infestation by *Sitotroga cerealella*, *Sitophilus oryza*, *S. granarius*, *Tribolium confusum* and *Cryptolestes pusillus* was noted by the writers as a common occurrence in farmers' bins in Montgomery County, Md., during the winters of 1921-22 and 1922-23.

The heating of grain caused by insect infestation was known many years previous to American observations. It should be recorded that in 1762 Duhamel du Monceau and Tillet, in discussing the Angoumois grain moth wrote (1):

The emergence of moths is usually preceded by a heating which spreads in the sheaves and piles of grain. Thermometers register 25° R. (89° F.) to 30° R. (100° F.) when the exterior air is only 15° R. (66.2° F.). The cold of autumn stops development and no more moths are seen until spring. Heat in the grain must be generated by the insects assembled in great numbers. Grain heavily infested becomes very warm. No heat was noticed in slightly infested grain, and the heat disappeared when most of the moths had emerged. Sometimes the heat passes promptly, at others it lasts for 3 or 4 weeks. It may result from a fermentation caused by the humidity that insects produce. It is certain that when the harvest is wet and warm rains follow, the piles of grain heat in a very short time and the insects breed rapidly. Without doubt, the heat is very favorable to the development of the moth, and the damp grain is more easily attacked by the insects. In 1760, when it was hot and dry, the people did not expect any insects, but they were very abundant, although the wheat did not heat noticeably, until mid-September. In 1761 the grain was very hot, its temperature being 53° R. (about 150° F.). Each year, even when harvests are dry, wheat heats independently of insects. However, the heat is always greater in infested wheat than in clean wheat, so that we can attribute it partly to the insects and partly to the humidity. When humidity causes an increased heat, everything is more favorable for the multiplication of the insects and when this multiplication takes place, the heat increases still more.<sup>7</sup>

The immediate interest of the writers has been centered in the possibility of reducing the temperature of heating grain to normal. In the references already quoted regarding the development of heat in grain due to insects, no mention is made of preventing the heating by remedial measures directed against the insects themselves, except that Back states (16, p. 23-24) that fumigation with hydrocyanic-acid gas kills *Bruchus quadrimaculatus*, "reduces the temperature of chick-peas to normal and stops spread." However, Herpin (2, p. 9) in 1860 stated that hot wheat infested with the Angoumois grain moth cools off in a few days after the insects are killed by asphyxiation, and this seems to be the only reference definitely answering the query "Will fumigation bring back to normal the temperature of grain which has heated as a result of insect attack?" The writers wish to record several opportunities given them to make observations upon this point.

During the summer of 1918, because of the congested warehousing conditions in New York City, one hundred and thirty-seven thousand 240-pound sacks of chick-peas, grown in Mexico, were held in six large warehouses in New Orleans. In October of that year the owners discovered that many sacks were heating badly because of infestation by *Bruchus quadrimaculatus*. Opportunity was given the writers to become personally familiar with the problem as presented in the various warehouses. Because of the demand for space resulting from war conditions, the sacks of seed had in many instances been stacked 20 deep. Heating sacks were found in all warehouses, and as the approaching cold of winter had no effect upon the heating sacks, it was recommended that the warehouses be fumigated with hydrocyanic-acid gas. In one tier of sacks temperature readings were made with ordinary soil thermometers on November 30 and December 1, with the results indicated in figure 2.

The temperature of the warehouse at the time these readings were made was 58° F. Uninfested sacks registered rather uniformly 60° F., while as noted in Figure 2, the temperature of the infested sacks ranged as high as 103° F. On December 1 the warehouse was fumigated with hydrocyanic-acid gas, using 2½ pounds of sodium cyanid per thousand cubic feet of space, this being the dosage desired by the owners. One week later, after the warehouse had been thoroughly ventilated and opened throughout the day for five days, readings were again made with soil thermometers. The warehouse temperature outside the sacks was 68° F. at 11 a. m. The temperatures secured for sacks numbered 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 13, 14, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, and 29, were 64°, 65°, 66°, 64°, 68°, 66°, 72°, 65°, 72°, 67.5°, 68°, 67°, 68°, 67°,

<sup>7</sup> Condensed translation, with temperatures in Fahrenheit added.

66°, 72°, 69°, 68°, 68°, 64°, 68°, 74°, 68°, 68°, 64.3°, and 70° F., respectively. While the warehouse temperature stood at 68° F. when these readings were in progress, the temperature of the warehouse over a 24-hour period was undoubtedly somewhat lower, and it was the belief of the writers that the temperature of the sacks was still falling slowly to about 60° F. No opportunity for further readings was given, and no record was made of the moisture content of the seed. The fumigations, conducted similarly in all six warehouses, was thoroughly effective and in each warehouse the heating in the sacks disappeared after the fumigations. This was naturally a matter of great concern to the owners, since the retail value of the seeds at this time was about \$5,000,000.

Studies were made during December, 1921, and January, 1922, of certain bins of wheat on farms near Washington. These bins were infested by the Angoumois grain moth, the rice weevil, and the flat grain beetle, the last two predominating. On December 21, when the barn temperature registered 27° F., the temperature of the wheat in one bin about 15 by 5 by 4 feet was 58° F. at its surface, 104° F. 6 inches below the surface, and 109° F. 2 feet below the surface. The upper layer of wheat for several inches from the surface was caked and mouldy. This wheat was fumigated with carbon disulphid on December 21 and left well covered with a tarpaulin until December 23, when the

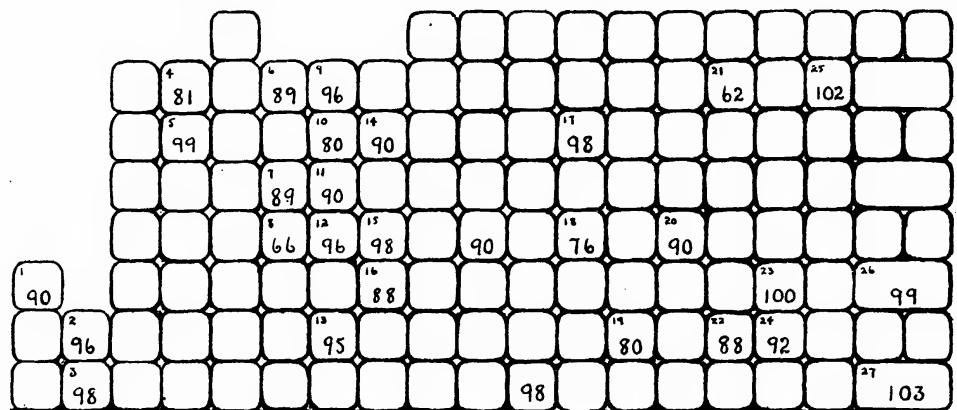


FIG. 2.—Diagram showing ends of 240-pound sacks of chick-peas arranged in single tier, with temperature in degrees Fahrenheit, the heat being due to infestation by *Bruchus quadrimaculatus*

tarpaulin was removed. Examination on December 23 showed the temperature at the bottom of the bin to be 76° F.; 2 feet from the surface at end of bin, 78° F.; and 2 feet from the surface in the center, 100° F. The tarpaulin appeared to be responsible for the slow fall in temperature up to December 23. On December 31 readings were taken which showed the temperature in the center at the ends to be 60° F., and in the middle of the bin 54° F. Final readings taken on January 4, 1922, gave the temperature as 40° F. for both the center and the ends of the bin.

In a second bin about 18 by 18 feet, in which the grain was not over 2 to 3 feet deep, the temperature due to the rice weevil and the flat grain beetle gave readings of 98°, 98°, 93°, 71°, 94°, 98°, 90°, 100°, 38°, 38°, and 38° F., respectively, according to the spot chosen for the reading. These temperatures were attained in wheat from 1 to 1½ feet below the surface at a time when the outside temperature was 30° to 40° F. In a third bin, where the wheat was spread to a depth of from 1 to 1½ feet on a floor beneath which was an open wagon shed there were spots of rice weevil infestation where the temperature ranged as high as 92° F. Other temperatures recorded for such shallow grain were 76°, 88°, 82°, 91°, 90°, 70°, 74°, 78°, and 45° F. Both of these last-mentioned bins were fumigated with carbon disulphid on January 11, 1922. Temperature readings were made on January 13 and January 20, with the results recorded in Table II.



TABLE II.—*Fall in temperature of grain following fumigation with carbon disulphid*

## BIN No 1

Tempera- tures before fumigation, January 11, 1922	Tempera- tures, January 13	Tempera- tures, January 20
°F.	°F.	°F.
100	60	40
90	50	40
98	56	40
94	56	40
71	50	40
93	50	40
98	56	40
98	58	40

## BIN No. 2

°F.	°F.	°F.
76	60	40
88	62	40
82	58	40
91	60	40
90	60	40
70	55	40
92	58	40
74	54	40
78	58	40
45	40	40

It should be recorded that a portion of the grain in the last-mentioned wheat was not fumigated, and while the temperature of the fumigated portion dropped to 40° F., as noted in Table II, the unfumigated grain continued heating for over a month until it was sold by the owner. Samples of wheat taken from this bin before fumigation showed the hottest wheat (91° F.) to contain a moisture content of 15.1 per cent; the cold, unheating wheat a content of 13.9 per cent. After fumigation, the grain which had been hottest gave upon analyses a moisture content of 14.4 per cent.

## CAUSE OF HEATING IN GRAIN

Factors causing heating of grain are not well understood. Enzymatic action and growth of molds such as *Aspergillus* are said to be associated with increasing temperature when no insects are present. Bailey and Gurjar state (13) in the conclusions to their report that the deductions from their investigations "support the findings of earlier investigations that spontaneous heating in damp grain is occasioned by the biological oxidation of dextrose and similar sugars, chiefly in the germ or embryo of the kernel." Dendy and Elkington (14) in 1919 published an article on the prevention of heating in wheat by means of air-tight storage, basing their work upon the assumption that the heating of the grain is confined to those portions to which air has free access, and not taking insects into consideration. The temperature of about 150° F. (53° R.) recalled by Duhamel du Monceau and Tillet in 1762 in their report on the Angoumois grain moth must have been caused by agencies other than insects, for grain pests are killed by temperatures above 125° F. It is believed by some that the heating of grain is sometimes started by insects and that their development makes it possible for other agencies not so well understood to gain a foothold and carry on the destruction. In discussing a paper by Doran (?), presented before the Washington Entomological Society in 1892, its author expressed the opinion that heating of

grain was due to the conversion by the insect at the time of transformation of a "certain amount of so-called 'latent heat' into sensible heat." In other words, that a certain amount of "heat energy" is expended in the operation. This seems especially probable owing to the more or less complete histolysis during the pupa stage. Dr. C. L. Marlatt (?), present at the meeting, was of the opinion that the heat was partly produced by fermentation. Austin (?) said that he thought the heat due to the chemical action of the forces setting up the fermentation with the grain, while Dr. E. A. Schwarz said (?) that the accumulation in numbers of insects produced considerable heat, and referred to the hibernation in crowds of *Megilla maculata*.

The heat produced by the honeybee cluster in winter has been well studied by Phillips and Demuth (11), who state that as the temperature of the air in the hive falls in winter the bees become less active until a certain critical temperature (about 57.2° F.) is reached, at which the bees undertake by muscular activity, not unlike that of shivering, to produce heat in order to keep warm. By such action the honeybees are able to maintain a temperature within their cluster of 86° to 95° F., when the outside temperature is 32° F.

The writers have been told by owners of grain elevators that in transferring grain from one bin to another, a transaction known as "running," they have observed hot spots in the grain at which there would be a "ball of insects." Because of the value of the grain, the necessity for prompt action on the part of the owner if heating grain is to be kept from spoiling, and the reticence of most owners to confide specific instances of difficulty to those who may inadvertently transmit trade information to competitors, it is extremely difficult to gain a first-hand knowledge of this so-called "balling-up" of grain pests at points in large elevators. While the writers are not prepared to state that grain pests do make a practice of congregating in cold weather to develop a temperature making possible their continued activity throughout the cold months, they believe that this does happen at times and that this phenomenon offers an interesting field for investigation. The coming together of large numbers of adult insects, as in the case of the bran in which Doran recorded finding approximately 1,500 beetles to the pound, might afford reason for believing that adult insects only are capable of starting an increase in the temperature of grain. Without denying entirely that adults alone are capable of initiating the heating process in grain, the writers are confident that in many instances the developing larvae are quite as important, if not more so.

That the immature stages of *Bruchus quadrimaculatus* must play a large part in the heating of chick-peas would appear from the data of Tables III and IV, in which is recorded the infestation of six samples of 50 seeds each, taken from sack No. 25 of figure 2, which at the time the samples were taken had in the center at 1 foot from the end a temperature of 102° F. These 300 seeds bore 2,174 unhatched eggs, 527 larvæ less than half grown, 453 larvæ one-half to full grown, 98 pupæ, 193 adults about to emerge, and showed indications of 98 adults already emerged (though not necessarily living) at the time of examination. Assuming that each sample of 50 seeds weighed 1 ounce (as one average sample actually did), sack No. 25 contained an infestation of 3,550,960 bruchids in various stages of development. Likewise, sack No. 7 (figure 2) with a temperature of 89° F., if it had an infestation indicated by the data in Table III, might have an estimated infestation of 1,286,640 *Bruchus quadrimaculatus* in all stages.

A study of the more detailed data on the infestation of Sack No. 25, as set forth in Table IV, will give the reader a very concise idea of the degree of infestation by *Bruchus quadrimaculatus*. No such complete data on infestation in relationship to heating of wheat as those here given covering the infestation of chick-peas had ever been presented. The writers present in Tables V and VI, as a report of progress, the rather meager data which they have.

TABLE III.—Data on the infestation of chick-peas by *Bruchus quadrimaculatus*

Sack number	Temperature of seeds	Sample No.	Number of seeds examined	Number of seeds infested	Number of Bruchus—								Total larvae, pupæ, and adults	Eggs unbatched
					Eggs	Emergence holes	Larvæ				Pupæ	Adults in seeds		
							Eighth grown	Quarter grown	Half grown	Full grown				
25.....	102° F.	1	50	47	549	11	16	6	19	77	31	51	200	
		2	50	47	598	13	8	13	33	53	26	43	176	
		3	50	49	619	22	97	14	35	39	10	25	220	
		4	50	48	523	17	59	26	16	52	11	30	194	
		5	50	48	548	24	24	11	35	56	19	37	182	
		6	50	49	706	11	174	79	30	8	1	7	299	
Totals	-----	-----	300	288	3,543	98	378	149	168	285	98	193	1,271	2,174
7.....	89° F.	1	50	50	308	32	2	6	30	111	70	41	260	
		2	50	49	298	32	3	0	8	93	60	32	196	
		3	50	47	262	32	8	1	5	85	43	25	167	
		4	50	45	175	37	6	0	7	58	48	17	136	
		5	50	50	296	23	3	1	19	100	49	11	183	
		6	50	47	286	6	22	15	31	63	20	4	155	
Totals	-----	-----	300	288	1,625	162	44	23	100	510	290	130	1,097	366

TABLE IV.—Infestation of 50 seeds from each of six samples <sup>a</sup> taken with grain-car probe from a 240-pound sack of chick-peas infested with *Bruchus quadrimaculatus* and registering 102° F.

Seed No.	Adults unemerged		Larvæ				Total number of insects in seed	Emergence holes						
	Pupæ		Full-grown	Half-grown	Quarter-grown	Eigh t grown								
SAMPLE NO. 1														
1.....	1					2	3	1						
2.....	5						5	2						
3.....	1					1	1	2						
4.....	1	2	2		1		6	2						
5.....					1	2	3							
6.....	1		1				2							
7.....	2	3	3				8	2						
8.....	2		2	3			7	1						
9.....	2		2			3	7							
10.....	1		1				2	1						
11.....	1	1	1				3							
12.....	1	1	6			2	10							
13.....	1	3	3				7							
14.....			3		1		4							
15.....	2	1	2				5							
16.....	1	1					2							
17.....			3	1			4							
18.....	2	2		1			5							
19.....						2	2							
20.....			3				3							
21.....	2	1					3							
22.....			2		2		4							
23.....			2	1			3							
24.....	3	1	3	1			8	2						
25.....						4	4							
26.....		1					1							
27.....	3			1			4	1						
28.....			2	1			3							
29.....				3			3							
30.....			2		1		3							
31.....	2						2							

Seed No.	Adults unemerged		Larvæ				Total number of insects in seed	Emergence holes						
	Pupæ		Full-grown	Half-grown	Quarter-grown	Eigh t grown								
SAMPLE NO. 1—Continued														
32.....		1						1	3					
33.....	2	1	2					5	1					
34.....	2	2	5	2				11	2					
35.....	4		1					5	1					
36.....								2						
37.....	1							1						
38.....	1	4	6					11	1					
39.....	2	1	3					6						
40.....								0						
41.....	1	1	4					6						
42.....	1	2	5	2				10						
43.....	1		3	3				7	3					
44.....		2	1					3						
45.....								0						
46.....	1							1						
47.....								0						
48.....	1							1					2	
49.....	4		1					5	1					
50.....	2	1	6	4				13						

SAMPLE NO. 2														
1.....	2		2	1				5						
2.....	2	1	2					5						
3.....				2				3						
4.....	1		2		1			5						
5.....	1	2	4					7						
6.....	1		2					3						
7.....		1						2	3					

<sup>a</sup> Samples 1 and 6 being at the extreme ends of the sack; samples 3 and 4 being nearest the center; all samples taken, naturally, along the central longitudinal axis of the sack.

TABLE IV.—Infestation of 50 seeds from each of six samples taken with grain-car probe from a 240-pound sack of chick-peas infested with *Bruchus quadrimaculatus* and registering 102° F.—Continued

Seed No.	Adults unemerged	Pupæ	Larvæ				Total number of insects in seed	Emergence holes
			Full-grown	Half-grown	Quarter-grown	Eighth grown		
SAMPLE NO. 2—Continued								
8			2	3			5	
9	2		4				6	1
10	2		2	1			5	1
11	2	1			2		5	1
12		1		1			2	1
13	1						1	1
14	1						1	
15		5	2				7	1
16	1						1	1
17	1						1	1
18	2	2	3		3		10	1
19	3						3	1
20	1						1	
21			3				3	
22				3	1		4	
23	1	1	3				5	
24			1				1	
25	1			4			5	
26							0	
27		1					1	
28				3			3	
29	2		3	5			10	1
30						2	2	
31						1	1	
32	2	2					4	
33	1		1	1			3	
34			2	2			4	
35	2	3					5	
36	1						1	
37				1			1	
38	1		1		1		3	
39	1	1	2				4	1
40			1	2			3	
41					2	1	4	
42		1	2				3	
43	2		1				3	
44			1				2	
45	2		2				4	
46							0	
47	1			2			3	
48	2	1	3	3			9	
49	1	1	2				4	
50	1	1	1				3	
SAMPLE NO. 3								
1	1					2	3	
2					3		3	1
3			2		1		3	1
4	1	1	2				4	3
5	1	1	1				2	
6	1	1	1				3	1
7	1	1	1				3	
8			1			1	2	
9				1		2	3	
10						2	2	
11	2					2	2	1
12	1					1	2	
13					2	1	3	1
14					1		5	
15							3	
16							3	
17							3	
18			2	8			10	
19		1	2	3			6	
20	1	1	2			5	9	
21	1		1	3			5	
22	2	2	4				8	2
23			1	3			4	
SAMPLE NO. 3								
24								
25		1						
26								
27								
28								
29								
30				4				
31	1		2	1				
32			3		2			
33	2		1	2				
34	1					8		
35						5		
36						4		
37			1	2				
38						5		
39					2	1		
40	1					2		
41	3		1	1				
42	1	1	2					1
43			1			3		
44	1					2		
45				1	1			
46	1							
47	1		1					
48	1		1					
49							0	
50		2	6			3	11	
SAMPLE NO. 4								
1		2						
2			2	2				6
3		1		3		2		4
4		2				3		5
5		3		1				4
6		1		2			3	6
7				2			3	5
8				3		2		5
9							1	1
10							1	1
11			3			7		10
12							4	
13							3	
14						3		
15							5	
16							3	
17							3	
18			1	3				4
19		2	1	3				6
20				2				4
21							3	
22							3	
23							4	
24						2	1	
25			1	1				
26							3	
27		3		4				7
28			2	1				3
29		3	1	2	1			7
30		1	2	1				3
31					2	1		
32					1	2		
33		3	1					4
34								0
35						2	2	
36		2	1	2				5
37		2			1			4
38				2	1			3
39				3	3			6

TABLE IV.—Infestation of 50 seeds from each of six samples taken with grain-car probe from a 240-pound sack of chick-peas infested with *Bruchus quadrimaculatus* and registering 102° F.—Continued

Seed No.	Adults unemerged		Larvæ				Total number of insects in seed	Emergence holes
	Pupæ	Full-grown	Half-grown	Quarter-grown	Eighth grown			
SAMPLE NO. 4—Continued								
40						1	1	
41		1				2	2	
42						1	1	
43	1					3	4	
44						2	2	
45			1	1		2	2	
46	3	2	7			12	0	
47								
48			3			3	3	
49						4	4	
50	1					1	1	1
SAMPLE NO. 5								
1					1	2	3	
2	2		3				5	
3			2		3		5	
4	1	1				3		
5	2	1	1	4			8	1
6	3	2	3				8	
7		2	3	1			6	
8	3		2				5	
9		1	2	1			4	
10				3			3	
11	2	3	6				11	
12			2	1			3	
13				3			3	
14	2			3			5	
15			1	2			3	
16		1	1	1			3	
17	2	3	2		3		10	
18	1					1	2	
19	1		2	2			5	
20						6	6	
21		1	1		2		4	
22			1				0	
23	1						1	
24							0	
25						1	1	
26	4		2				6	
27		1	1				2	3
28	2		4				6	1
29			1	1			2	1
30	2						2	
31			4	2			6	
32	2	1		5			8	
33							0	
34	1				2		3	
35						4	4	
36	1					2	3	
37	1		2				3	1
38			2	1			3	
39	2						2	1
40		1	4	1			6	
41	1		1	1			3	1
42	1	1	4	2			8	
43		1		4			5	
44						2	2	
45				2			3	
SAMPLE NO. 5—Continued								
46						2	2	
47		1		4			5	1
48	1			4			5	
49	1	2		3			6	
50	1	4		3			8	
SAMPLE NO. 6								
1							8	8
2		1		2	2		2	7
3				2	2		4	6
4				2	2		5	7
5		2		1			1	4
6		1					4	5
7		2					5	7
8							2	2
9							6	6
10							2	2
11				2			3	5
12							6	6
13							3	3
14		1		3			3	7
15							3	3
16					3	2	3	8
17					3	3	2	8
18						4	5	9
19						4	2	6
20						6	6	12
21						5	5	10
22					3		2	5
23				1			8	9
24		1					5	6
25						3	4	7
26							6	6
27					1		4	5
28						4		5
29							3	3
30						8		8
31							6	6
32						9		9
33						4	1	5
34		1		1	8			10
35						2	8	10
36							13	13
37				1	2		9	12
38						8		8
39							4	4
40							9	9
41							8	8
42							3	3
43								0
44						4		4
45						5		5
46						4		4
47							2	2
48				1	1	2		4
49						2		2
50						1	7	8

The data of Table V on the relative abundance of the rice weevil and the flat grain beetle in probes taken at varying points in a wheat bin having warm spots indicate, as one might expect, a large number of insects present in the warm spots. The data are unsatisfactory in that there are not enough records of the abundance of insects in grain having temperatures below 70° F., for at temperatures between 70° and 90° F. grain pests are thoroughly active.

TABLE V.—Number of *Sitophilus oryza* and *Cryptolestes pusillus* found in one-ounce samples taken at four depths in bin with varying temperatures <sup>a</sup>

Point in bin	Temper- ature at center	Number of insects in sample from—								Total insects for probe
		Bottom of bin		1 foot from bottom of bin		1½ feet from bottom of bin		6 inches from top of grain		
		<i>Sito- philus oryza</i>	<i>Crypto- lestes pusillus</i>	<i>Sito- philus oryza</i>	<i>Crypto- lestes pusillus</i>	<i>Sito- philus oryza</i>	<i>Crypto- lestes pusillus</i>	<i>Sito- philus oryza</i>	<i>Crypto- lestes pusillus</i>	
	° F.									
A.....	38	1	0	0	0	0	0	1	0	2
B.....	38	0	1	3	0	1	3	0	0	8
C.....	38	0	0	1	2	0	1	0	0	4
D.....	100	1	2	14	12	17	15	4	2	67
E.....	90	6	18	15	35	14	22	6	10	126
F.....	98	0	0	2	6	10	30	6	8	62
G.....	94	6	12	5	5	3	13	10	20	74
H.....	71	8	5	18	14	21	5	7	3	81
I.....	93	3	4	8	13	16	27	6	2	79
J.....	98	6	11	6	9	9	9	8	0	58
K.....	98	0	4	4	5	17	16	9	0	55

<sup>a</sup> Reading taken where wheat was about 2½ feet deep.

The data of Table VI are of importance in that they give one an idea of the high percentage of wheat kernels actually found infested when examined for immature stages beneath the compound microscope.

The writers can not help but conclude from their observations that the insects themselves are capable of developing heat in grain in storage. It does not seem probable that there is much truth in the often expressed idea that the heating of insect-infested grain is usually due to a fermentation set up by the absorption of moisture by the faeces of the insect. The writers have seen seeds (wheat, beans, and chick-peas) badly damaged and heating because of insects, that were leathery rather than brittle because of the moisture apparently absorbed by the seeds, or the faeces in the seeds. Thus badly infested seeds in sack 25 of Table III could be pressed flat without breaking because, as the writers thought, of the humid conditions produced by the infestation. Yet the fact remains that the heat left not only this sack, but all the sacks of chick-peas fumigated with hydrocyanic-acid gas in the warehouses, and the wheat fumigated with carbon disulphid in farmers' bins. It may be recalled, also, that Richardson (5) and Duhamel du Monceau and Tillet (1) have said that the heating of seeds ceases often with the passing of the infestation (usually after the seeds have been rendered valueless as food and filled with excreta). Unless it can be proved that bacteria or other organisms causing heat are killed by fumigation with hydrocyanic-acid gas or carbon disulphid, there seems no other conclusion than that the activity of certain stored-product pests is directly responsible for the development of heat in grain in certain instances and that fumigation will kill such heat and permit the grain to return to normal temperature.

TABLE VI.—Percentage of wheat kernels infested and number of adult insects found free in ounce samples in bin the wheat of which registered 58° F. at surface, 104° F. 6 inches below surface, 109° F. 2 feet below surface, and 88° F. at bottom of bin about 4 feet below surface

Depth at which sample was taken	Location of probe											
	At end of bin				At center of bin				At end of bin			
	Per cent of ker- nels in- fested	Number of speci- mens of—			Per cent of in- fested ker nels	Number of speci- mens of—			Per cent of ker- nels in- fested	Number of speci- mens of—		
		Oryza	Pusil- lus	Para- sites		Oryza	Pusil- lus	Para- sites		Oryza	Pusil- lus	Para- sites
6 inches .....	92.1	2	6	3	93.2	2	4	2	88.9	6	3	2
12 inches .....	94.3	27	12	0	94	7	14	8	93.9	7	7	3
18 inches .....	90.9	7	12	3	96.4	7	15	3	90	6	8	3
24 inches .....	92.3	3	17	13	93.7	4	4	6	96.5	3	3	0
30 inches .....	91.8	4	9	3	91.2	4	3	3	92.3	3	5	0
36 inches .....	93.4	3	5	1	90	1	4	2	84.2	3	3	6
42 inches .....	95.5	24	6	6	91.4	2	10	2	89.8	4	10	9
48 inches .....	90	4	1	0	91.1	1	7	2	94.9	32	14	9

## DESTRUCTION OF HEAT BY FUMIGATION OF MOST PRACTICAL VALUE

Whatever may be the reader's conclusion regarding the actual cause of heating in insect-infested seeds, there can be no doubt as to the value of any treatment that will reduce the temperature of the seeds to normal. The placing of heating seeds in cold storage will not prevent insect activity if the seeds are sacked or placed in bulk and are already heating when placed in cold storage. Cowpeas infested with *Bruchus quadrimaculatus* and beans infested with *Bruchus obtectus* continued to heat for several months when placed in cold storage at 32° F., according to the experience of several operators of storage plants.

In ordinary storage where seeds are stacked as indicated in figure 2, the heating seeds radiate sufficient heat to raise the temperature appreciably in the spaces between the sacks. Thus, with a warehouse temperature of 58° F. the space between sacks No. 22, 23, and 24 (sacks with temperatures of 88°, 100°, and 92° F., respectively) registered 78° F. In many instances the temperature in the spaces between or touching heating sacks was raised to 70° and 72° F. Bruchids at 58° F. are not capable of mating or spreading, but at temperatures above 70° F. they reproduce actively and bring about a spreading of the infestation at a season of the year when the owner normally expects protection from insects due to the action of cold weather. The same holds true in wheat bins on the farm, even in bins of wheat where the seeds are not deeper than 1 to 2 feet. The heating permits feeding and reproduction to progress even in the dead of winter. A thorough fumigation with hydrocyanic-acid gas or carbon disulphid kills the insects or a sufficient number of them so that those that remain can not maintain a condition favorable to the production of progressive destruction.

## SUMMARY

It is well known that the heating of grain may be caused by a high moisture content. Spontaneous heating in damp grain is occasioned by the biological oxidation of dextrose and similar sugars, chiefly in the germ or embryo of the kernel. Enzymatic action and growth of molds have been said to be associated with increasing temperatures when no insects are present. With heating due to causes other than insect infestation this paper does not deal.

It has been assumed by certain grain authorities that when wheat shows a moisture content of less than 14.5 per cent it is likely to remain in condition if

given proper storage and kept free of insects. However, when insects are present grain has been known to heat when the moisture content is as low as 12 per cent. A study of 15,000,000 bushels of wheat by the Bureau of Markets indicated that when the moisture content was as low as 11.3 per cent to 12 per cent heating due to insect infestation occurred, and resulted in the conclusion, by eliminating factors of moisture, damage, and time in storage, that the rising temperatures were caused by insect infestation.

The heating of grain and grain products when insects are present has been noted before. The writers have known chick-peas in 240-pound sacks to heat as a result of attack by *Bruchus quadrimaculatus*, the temperature rising to as high as 103° F. when the normal temperature was 58° F. Wheat stored in farmers' bins and well infested by *Sitophilus oryza* and *Cryptolestes pusillus* was found to have developed a temperature as high as 109° F. when the normal temperature was 27° F. Wheat in shallow bins or on barn floors and stored in piles ranging in depth from 1 to 3 feet was found at times heating if badly infested by the two pests just mentioned and the Angoumois grain moth. It is not difficult to find many instances of heating in cereals and such cereal products as animal feeds.

The cause of heating when insects are present in numbers is not clearly understood. Probably the most prevalent opinion is that heating is due to fermentation started by the attraction of moisture to the faeces of the insects. Others have given as the cause the mechanical friction due to the insects' feeding. It is known that the honeybee cluster in winter can develop by muscular activity not unlike shivering a temperature in the hive of 86°–95° F. when the normal temperature is but 32° F. Grain dealers claim that insects do congregate in large numbers in spots in grain in elevators and that such "balls of insects" are responsible for starting grain heating. As many as 1,500 *Carthartus cassiae* beetles have been recorded in 1 pound of bran heating at a temperature of 95° F. The writers estimated one 240-pound sack of chick-peas with a temperature of 102° F. to contain 3,550,960 Bruchids in various stages of development; the sacks not heating, and having a normal temperature of 58° F., showed no infestation. It is undoubtedly true that when grain heats badly as a result of insect infestation the insects are present in great abundance.

The fumigation of grain, heating as a result of insect infestation, with either carbon disulphid or hydrocyanic-acid gas brings about a fall in the temperature of grain to normal. This was found true in the fumigation of one hundred and forty thousand 240-pound sacks of chick-peas stored in six warehouses and containing hundreds of heating sacks. It was also found true in the fumigation of heating wheat in farmers' bins.

The destruction of heat in grain during winter is of great importance, particularly on the farm. In ordinary storage heating seeds radiate sufficient heat to raise appreciably the temperature in the spaces between the sacks in the case of sacked seeds. The temperature, which was 58° F. between uninfested sacks of chick-peas, was raised to as high as 70° to 78° F. between infested and heating sacks, with the result that the Bruchids, dormant at 58° F., were able to multiply and actively spread the infestation from the infested and heating sacks. The activity of pests in stored corn and wheat is in like manner made possible by heating. Heating permits feeding and reproduction to progress even in the dead of winter. Fumigation does what cold weather will not do; it kills the heat and reduces the temperature of the seeds to normal and thus prevents progressive destruction.

Unless it can be proved that bacteria, molds, or other agencies causing heat are killed by fumigation with hydrocyanic-acid gas or carbon disulphid, the writers conclude that the activity of certain stored-product pests is directly responsible for the development of heat in grain in certain instances and that fumigation will kill such heat and permit the grain to return to normal temperature.



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# THE NITRIFICATION OF PHOSPHORUS NITRIDE <sup>1</sup>

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Phosphorus nitride has recently received some attention as a possible fertilizer product from the fixation of atmospheric nitrogen. A survey of the literature furnished no direct information as to the fertilizer value of this material. As a result of vegetation experiments, Stutzer <sup>2</sup> found that silicon nitride and other nitrides were of little or no value as fertilizers. The article does not state whether phosphorus nitride was included in this work. In order to secure definite information on phosphorus nitride, the experiment reported below was made. This work was based upon the well-known fact that materials which do not nitrify readily do not serve as satisfactory nitrogenous fertilizers, although in unusual cases it is possible that such materials may have some plant food value.

The phosphorus nitride used in this study was prepared by the action of ammonia on phosphorus pentasulfide and contained 40.03 per cent nitrogen. The purity of the sample on the basis of the formula  $P_3N_5$  was, therefore, 93.3 per cent. It was shown that the sample contained an excess of phosphorus and it is probable that this element accounts for the greater part of the remaining 6.7 per cent. It was probably present largely as free phosphorus, but some compounds of the type PNS and PNO may have been present also.

The experiment was made with a Susquehanna loam soil, using 100-gm. samples in 300-cc. beakers. In order to provide optimum conditions for nitrification, 0.5-gm. calcium carbonate was added to each soil sample. The phosphorus nitride and the ammonium sulphate used for comparison, as well as the calcium carbonate, were thoroughly mixed with the dry soil and 21 per cent distilled water added. The soil samples were maintained at room temperature during the course of the experiment and any water lost by evaporation was restored frequently. At intervals, nitrate determinations were made on a group of samples, using the phenoldisulphonic acid method. These results are given in Table I.

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<sup>1</sup> Received for publication Apr. 28, 1924—issued November, 1924.

<sup>2</sup> STUTZER, A. UEBER NITRIDE IN IHREN WIRKUNGEN AUF PFLANZEN. (Abstract) Chem. Ztg. 34: 1015. 1910.

TABLE I.—Comparison of nitrate formation in soils receiving phosphorus nitride and ammonium sulphate

Treatment	Mgm. N added per 100 gm. of soil	Nitrate nitrogen	Average	Increase over control	Nitrate nitrogen	Average	Increase over control
		19 days			35 days		
		Mgm.	Mgm.	Mgm.	Mgm.	Mgm.	Mgm.
Control.....	0	<div>6.58</div> <div>7.14</div>	6.86		<div>7.44</div> <div>7.88</div>	7.66	
Ammonium sulphate.....	5	<div>10.00</div> <div>10.42</div>	10.21	3.35	<div>11.33</div> <div>11.84</div>	11.59	3.93
Do.....	10	<div>10.00</div> <div>10.00</div>	10.00	3.14	<div>16.54</div> <div>17.37</div>	16.96	9.30
Do.....	20	<div>9.62</div> <div>9.26</div>	9.44	2.58	<div>26.05</div> <div>26.72</div>	26.39	18.73
Phosphorus nitride.....	5	<div>7.58</div> <div>7.35</div>	7.47	.61	<div>8.40</div> <div>8.40</div>	8.40	.74
Do.....	10	<div>7.14</div> <div>7.58</div>	7.36	.50	<div>8.14</div> <div>8.14</div>	8.14	.48
Do.....	20	<div>7.35</div> <div>7.35</div>	7.35	.49	<div>8.14</div> <div>8.02</div>	8.08	.42
		56 days			75 days		
Control.....	0	<div>8.68</div> <div>9.30</div>	8.99		<div>8.40</div> <div>8.68</div>	8.54	
Ammonium sulphate.....	5	<div>14.89</div> <div>14.08</div>	14.48	5.49	<div>14.08</div> <div>13.36</div>	13.72	5.18
Do.....	10	<div>18.61</div> <div>19.30</div>	18.96	9.97	<div>18.61</div> <div>19.30</div>	18.96	10.42
Do.....	20	<div>28.94</div> <div>27.42</div>	28.18	19.19	<div>28.16</div> <div>27.42</div>	27.79	19.25
Phosphorus nitride.....	5	<div>8.98</div> <div>8.40</div>	8.69	-.30	<div>8.40</div> <div>8.54</div>	8.47	-.07
Do.....	10	<div>8.98</div> <div>8.68</div>	8.83	-.16	<div>8.54</div> <div>8.68</div>	8.61	.07
Do.....	20	<div>8.98</div> <div>8.98</div>	8.98	-.01	<div>7.89</div> <div>8.14</div>	8.02	-.54

It will be observed from the table that the addition of phosphorus nitride to soil, maintained under optimum conditions for nitrification, did not markedly affect the nitrate content. During the first month there was a slight increase but later a decrease in nitrates. This initial slight increase may have resulted from the nitrification of the phosphorus nitride or, on the other hand, this material may have merely stimulated the nitrification of the soil organic matter. It is impossible to state which of the two explanations is the correct one. However, it is certain, judging from the limited amount of data here presented, that the nitrogen in phosphorus nitride is not readily converted into nitrates and since plants for the most part require nitrate nitrogen for their best growth, it is very improbable that phosphorus nitride would supply that demand.

# AECIAL STAGES OF THE LEAF RUSTS OF RYE, PUCCINIA DISPERSA ERIKSS. AND HENN., AND OF BARLEY, P. ANOMALA ROSTR., IN THE UNITED STATES<sup>1</sup>

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## INTRODUCTION

In 1918, investigations on the leaf rusts of rye, barley, wheat, corn, and related grasses were begun by this laboratory in cooperation with the Office of Cereal Investigations, United States Department of Agriculture. Since it has been shown by Stakman (11)<sup>2</sup> and others that, as in northern Europe, *Puccinia graminis* Pers., the stem rust of wheat, oats, barley, rye, and related grasses, can be controlled in the northern United States by eradicating its alternate host, the common barberry, *Berberis vulgaris* L., it seemed important to determine the aecial hosts of the leaf rusts in this country and the rôle they may perform in propagating these rusts. In a previous paper, the writers (5) already have reported on the alternate host of the leaf rust of wheat, *P. triticina* Erikss., and at this time are presenting the results of similar studies on the leaf rusts of rye and barley.

## LEAF RUST OF RYE, PUCCINIA DISPERSA

DeBary (3) established the fact that *Anchusa arvensis* and *A. officinalis* are aecial hosts of *Puccinia dispersa*. This relationship was determined by inoculating the leaves of *Anchusa* with the basidiospores of the rust fungus. The resulting infection produced aeciospores which, when applied to the leaves of rye plants, produced infection and subsequent development of urediniospores.

When basidiospores of rye leaf rust were sown on *Berberis vulgaris* L., *Rhamnus frangula* L., *R. cathartica* L., *Ranunculus acris* L., *Ranunculus bulbosus* L., *Leonodon taraxacum* L. (*Taraxacum officinale* Weber), and *Urtica dioica* L., no infection resulted. Nielson (9) later obtained infection on rye following inoculation with aeciospores from *Anchusa officinalis*. Plowright (10) also obtained aecia on *Anchusa arvensis* by placing rusted rye straw near that host. Eriksson (4) was able to infect *Anchusa arvensis* and *A. officinalis* with *Puccinia dispersa* on rye, but obtained no infection on *Myosotis alpestris* F. W. Schmidt, *Symphytum asperum* Lepechin (*S. asperrimum* Donn), and *Pulmonaria officinalis* L. and only pycnia on *Nonnea rosea* Link. Sowings of aeciospores from *Anchusa* produced uredinia on rye but not on the other inoculated grass hosts. Klebahn (6) reports on similar experiments in which aecia were obtained on *Anchusa*. Uredinia also were obtained on rye when inoculated with aeciospores from *Anchusa*. In the

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<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 1126.

United States, Arthur (1, 2) secured infection on *Lycopsis arvensis* L. (*Anchusa arvensis*) with *Puccinia dispersa* from rye. A few pycnia developed in one instance and numerous pycnia and a few aecia in another. He states that—

It is possible, yet scarcely probable, that the explanation (of this weak development of aecia) lies in the lessened vigor of the American rye rust, which is propagated by repeating spores alone, the aecial hosts being practically wanting in America.

In Europe, the leaf rust of rye is characterized by several workers as having teliospores which develop and germinate during the same season. Eriksson (4) especially has emphasized this point and states that in Sweden the teliospores begin to germinate as soon as they mature, that is, about the middle of July, and cease germinating when kept out of doors during the winter. The aecia were produced from the beginning of August to the end of September. Although in Eriksson's experiments the teliospores did not germinate after overwintering in the open, he found they would remain dormant until spring if kept indoors. Most of the other European writers on rusts appear to agree with these statements of Eriksson. DeBary (3), however, who originally discovered the connection, states that the teliospores germinated after overwintering. Klebahn (6) questions whether this was out of doors, pointing out that DeBary does not state the manner in which they were overwintered. DeBary (3), however, does state that aecia are to be found throughout the year from spring to late summer and in mild winters even in January.

#### GERMINATION OF THE TELIOSPORES

During the winter of 1918-19, a number of collections of telia of the leaf rust of rye were wintered along with those of the leaf rust of wheat and were tested for germination in the spring of 1919. One collection of the leaf rust of rye germinated in April. Since, as stated above, the teliospores of the leaf rust of rye are generally believed to germinate only in the late summer and fall of the year in which they are formed, all the collections of telia of this rust, made in the spring and summer of 1919, were tested for germination beginning July 15, and at intervals of two to four weeks until April 7, 1920.

Each collection was tied in a coarse cheesecloth bag and hung on the east side of a building about 2 feet from the ground. All the material was brought into the greenhouse two days before testing and placed on moist sphagnum for that length of time. The test for germination was made by scraping some of the teliospores from the rye leaves and placing them in a hanging drop on the bottom of an inverted Syracuse watch glass. These drops were then examined after 12 hours.

Twenty collections of teliospores obtained from the States of Indiana, Georgia, Delaware, New York, Wisconsin, Minnesota, Nebraska, Kansas, Washington, Oregon, and California were thus wintered and tested. Of these, 15 showed germination at some time during the test. Germination first took place on August 19 in 2 collections, and between that date and November 25, 10 additional collections showed germination. All these collections and 2 others continued, for the most part, to germinate during December. During January, 11 of the 14 still showed some germination. In February there apparently was a sharp falling off in the number of collections showing germination, only six showing germination during that period. In March, another collection was added to the list for the first time. On April 7, when the material was last tested, five cultures still showed germination. One of these five was the collection which germinated on August 19 of the preceding summer. This culture showed germination on 9 consecutive occasions during the winter, another 10, a third 7, a fourth 6, and the other, which first showed germination in April, germinated twice.

These results are the more striking when one considers that the teliospores were subjected to sudden and extreme temperature changes, from low winter temperature to the warm temperature of the greenhouse. They then were placed out of doors and again suddenly subjected to low temperatures and the drying effects of freezing. Such treatment certainly would be expected to be fatal to any except the most hardy spores. This may explain why some of the collections did cease to germinate before spring. From the information now available, it would appear that in this country the teliospores of the leaf rust of rye, as a general rule, are capable of germinating whenever conditions are favorable either in the fall or, surviving the winter, in the spring, or both. The repeated germination of some of the collections would indicate that differences existed among the teliospores as to dormancy, otherwise one would expect all the spores to germinate when germination was first observed.

It is difficult to draw conclusions with reference to a rust of this type in which the telia long remain covered by the epidermis, as the length of time the epidermis remains over the telium may prove to be a factor. It is well known, however, that in some species of rusts, especially short-cycled species of *Puccinia*, there are to be found in the same telium spores which germinate at once and others which germinate only after a considerable resting period.

What the significance of the difference in action of the collections may be is difficult to say. While most of the collections which germinated in the fall continued to germinate until well into the winter, one showed germination only on September 12, and, although tested 13 times during the winter and spring, gave no more evidences of germination. Four others germinated only up to January 5. On the other hand, one collection gave no germination until March 8, although tested previously 10 times throughout the fall and winter. It is possible that in this country the leaf rust of rye may be made up of different strains differing, among other things, in the length of time which it takes to mature their teliospores and in the ability of these to overwinter. On the other hand, these differences may be due largely to environmental conditions under which the spores developed. These are questions which are difficult to solve as the leaf rust of rye, unlike some of the leaf rusts of native grasses, does not form its teliospores upon seedlings, and the time and care which would be necessary to grow different pure cultures of leaf rust of rye on maturing plants under controlled conditions has made it thus far impracticable to attempt to investigate this phase.

The writers' observations to the effect that all the teliospores of *Puccinia dispersa* necessarily do not germinate in the late summer and fall, but some at least may remain dormant during the winter, support the original statements of DeBary (3) as to the germination of the teliospores of this rust after overwintering, which have been somewhat questioned. It always has been difficult to determine how the aecial infection occurring in the fall can figure importantly in the winter survival of the rust. Certainly if the rust should not hibernate in the uredinal stage on the rye plant itself, the autumnal aecia can not be a factor in its survival. On the other hand, under such conditions, those teliospores which remain dormant until the next spring may be of importance in regions where the rust does not overwinter on rye itself and the aecial host is present.

From still another point of view, it is interesting to note the germinative ability of the teliospores of *Puccinia dispersa* subsequent to their winter survival. Eriksson was influenced to separate the leaf rust of wheat as a species, *Puccinia triticina*, from the leaf rust of rye largely because the former, unlike the latter, was unable to infect *Anchusa* and because the teliospores of the leaf rust of wheat germinated only after overwintering, while those of rye germinated shortly after harvest and did not overwinter. Here it may be pointed out that during the

winter of 1919-20, the authors observed that a number of collections of teliospores of the leaf rust of wheat were germinable in early December and continued to germinate until spring in a manner similar to those of *P. dispersa*. The distinction between these species on the basis of difference in time of germination of their teliospores therefore is not as sharp as it first appeared.

Klebahn (7) has shown that teliospores which usually are known to germinate only after the close of the winter season may be made to germinate by alternate drying and wetting. Recently, Maneval (8) also has shown for a number of such rusts that the teliospores will germinate if alternately wet and dried or soaked long enough. However, it probably is true that the teliospores of *P. triticina* generally germinate only after hibernation while those of *P. dispersa* usually are capable of germinating in the fall of the same year in which they are formed. However, they may retain this ability throughout the winter. Varying climatic conditions in different years, however, may tend to level such a distinction.

#### INFECTION OF ANCHUSA

The above collections, on being found germinable, were sown on potted plants of *Anchusa officinalis* grown in the greenhouse from seed obtained from Prof. Ed. Fisher of Berne, Switzerland. Of the 15 collections germinating only 5, however, produced infection. Of these 5, only 2 produced both pycnia and aecia, while the other 3 developed pycnia only. Aeciospores from the 2 plants showing aecia were sown on seedling plants of Rosen rye, producing uredinia typical of *Puccinia dispersa*. The 5 collections which gave infection were obtained from Mt. Vernon, Ind., Madison, Wis., Manhattan, Kans., Kearney, Nebr., and Seattle, Wash. These were the same collections which still showed germinating teliospores on April 7. Each of these collections, however, was sown a number of times without obtaining infection. This was especially true during the fall and winter, most of the infections being obtained in the spring, as happened in both instances of aecial production. Apparently the reason for this was the condition of the *Anchusa* plants, for they developed little new growth in the greenhouse during the fall and winter, and many of the older leaves died. With the advent of spring, new growth took place and more abundant infection resulted. In consequence, no very definite conclusions can be drawn from the negative results obtained from the other 10 collections as they germinated only at times when the *Anchusa* plants were in poor condition. It is evident, however, that the teliospores of *P. dispersa* which overwintered were able to infect *Anchusa*, thus establishing their identity with the species *Puccinia dispersa* as distinguished by Eriksson. But for this fact, the overwintering of the teliospores in the absence of infection of *Anchusa* would have indicated the possibility of their having belonged to another race or species of leaf rust on rye. By the close of winter, such a small amount of material was left that no attempt was made to inoculate plants of *Anchusa* in the field.

Although infection was not obtained to any extent in the greenhouse in the fall, mulching of *Anchusa* plants in the field, where they were in good growing condition, resulted in abundant production of aecia. On August 12, 1922, rye straw from the Agronomy plats, heavily covered with telia of the leaf rust of rye, was used to mulch vigorously growing plants of *Anchusa officinalis*. The summer of 1922 was very dry and no rain fell until August 23. On August 31, numerous yellowish spots containing pycnia were noted scattered over the leaves, petioles, and young flower parts. Aecia appeared in these areas in considerable abundance in about a week (Pl. 1, A). Again, on September 22, shortly following a rain, new infected areas containing pycnia were found scattered over the plants. Aecia also followed in these areas. With the fall rains, infection was more or less

continuous. On November 10, when last observed, the plants were covered with a heavy development of aecia and, even then, many newly-formed pycnia were evident. Although the rusted straw remained as a mulch throughout the winter, and the *Anchusa* plants produced vigorous growth the following spring, no infection was observed. In the fall of 1923, the same plants again were mulched with rye straw laden with teliospores of *Puccinia dispersa*. No infection resulted. This would indicate that, if its aecial host be present, the leaf rust of rye occasionally may produce its aecia in abundance in this country in late summer and fall just as it does in Europe. Also the indications are that the absence of its aecial host apparently has not destroyed in any way the inherent ability of the rust to produce aecia.

At the time *Anchusa officinalis* was mulched with rye straw, other boraginaceous species in close proximity also were mulched with the same material in order to determine whether the rust might not have still other aecial hosts. These Boraginaceae were *Nonnea rosea* Link, *N. lutea* DC., *Cerinthe minor* L., *C. major* L., *C. alpina* Kit., *Lappula echinata* Gilib. (*Myosotis lappula* L.), *Myosotis palustris* Hill, *M. arvensis* (L.) Hill, *M. alpestris* F. W. Schmidt, *M. virginica* (L.) BSP., *Symphytum asperum* Lepechin (*S. asperrimum* Donn) and *Pulmonaria officinalis* L. All these plants were exposed to infection equally with the plants of *Anchusa officinalis*, but in only one case was there any indication of infection. One plant of *Nonnea rosea* developed a few pycnia but the infection proceeded no further. This additional link in evidence points to the identity of the leaf rust of rye in this country and in Europe, for Eriksson (4) in Sweden found that *Myosotis alpestris* and *Symphytum asperrimum* were immune, while pycnia developed on *Nonnea rosea*.

Earlier in July, 1920, a new host for the leaf rust of rye was discovered in *Anchusa capensis* Thunb. This had been planted as a border plant around flower beds near the horticultural greenhouses of Purdue University. The plants showed a fairly heavy development of aecia (Pl. 1, B) the spores of which, when sown on rye, produced typical uredinia of the leaf rust of rye. As this sporulation occurred in advance of the application of the straw mulch, the infection must have started from a small plot of rye to the north, or perhaps from straw contained in the manure used in fertilizing the bed. This seems to be the only record of the occurrence of aecia of the leaf rust of rye in this country except where *Anchusa* plants had been planted and mulched for the purpose of obtaining the aecia. Flowering specimens of this species of *Anchusa* were sent to Prof. B. L. Robinson of the Gray Herbarium, Harvard University, who kindly had them compared with an isotype of *Anchusa capensis* and reported that they appeared to be identical.

It is not probable, however, that the aecial hosts of the leaf rust of rye are of much importance in this country at the present time. The aecia, with one exception, as stated above, have only been found where *Anchusa* plants have been mulched with rye straw for the purpose of producing the aecia. *Lycopsis arvensis*, presumably the *Anchusa arvensis* of northern Europe, occurs in North America from Nova Scotia to Ontario, Minnesota, Pennsylvania, and Virginia. So far as we have been able to determine, aecia of *Puccinia dispersa* have never been collected on this species. Since *Puccinia dispersa* overwinters fairly commonly in the uredinal stage on rye, the rust maintains itself without the aecial stage.

It hardly would appear probable that, were the development of the aecial stage in this country to occur in the fall, as it does in Europe, conditions affecting maintenance of the rust would change to any extent. The rust would have to survive the condition either in the aecial stage on *Anchusa* or, what is more probable, pass



over to rye and survive in the uredinial stage on that host. As volunteer rye in the eastern United States usually is heavily rusted in the fall, in the absence of *Anchusa* it seems improbable that the presence of the aecial stage would influence the situation to any extent. If the teliospores commonly retain their germinability until spring, *Anchusa* species may be of some importance in that they may serve as additional centers for spread of the rust. Our observations are not extensive enough to warrant drawing any conclusions as to this possibility, the general situation in Europe indicating its rare occurrence in the spring, even there.

### LEAF RUST OF BARLEY, *PUCCINIA ANOMALA*

The aecial stage of the leaf rust of barley, *Puccinia anomala* Rostr. (*P. simplex* [Körn.] Erikss. and Henn.), was unknown until Tranzschel (12), working in Russia in 1914, sowed teliospores on plants of *Ornithogalum umbellatum* L., *O. narbonense* L., *Muscari botryoides* (L.) Mill., *M. tenuiflorum* Tausch, *Scilla sibirica* Andr., and *Allium angulosum* L. Many aecia developed from this sowing on *Ornithogalum umbellatum* and a smaller number on *O. narbonense*. The other plants remained uninfected. Aeciospores from *O. umbellatum* were sown upon *Hordeum vulgare* L., producing urediniospores and teliospores. We have not been able to find records to show that these results have been repeated elsewhere.

Barley straw, heavily laden with teliospores of the leaf rust of barley, was collected at Washington, D. C., Blacksburg, Va., and Mt. Vernon, Wash., in the summer of 1921. Part of each of these collections was wintered in a similar manner to that described for rye, and part was used to mulch small areas where bulbs of the Star-of-Bethlehem, *Ornithogalum umbellatum*, had been planted. The collections of telia, when brought into the greenhouse on March 24, 1922, were found to germinate and were sown on Star-of-Bethlehem plants growing in pots. Infection was obtained in each case, pycnia appearing April 4, followed by aecia April 18 (Pl. 1, C). From these cultures, aeciospores were sown on barley, producing uredinia typical of *Puccinia anomala*. The groups of plants of Star-of-Bethlehem, mulched in the field with straw of each of these collections, also showed infection, pycnia appearing about April 15, followed by aecia. On May 18, uredinia developed on barley sown near the aecia-bearing *Ornithogalum* plants.

During the summer of 1922, barley straw bearing telia of *Puccinia anomala* was collected at Lafayette, Ind., and used to mulch *Ornithogalum umbellatum* in the field. On April 17, 1923, pycnia were noted upon these plants, followed on May 5 by aecia.

*Puccinia anomala* more nearly resembles a *Uromyces* than a species of *Puccinia*. The teliospores are for the most part one-celled. Indeed, in some collections considerable search is necessary in order to find the two-celled spores. According to usage, however, the presence of these few two-celled teliospores places the species in the genus *Puccinia*. A very similar rust, *Uromyces hordei* Tracy, is found in southwestern United States upon a wild barley, *Hordeum pusillum* Nutt. This, however, produces only one-celled teliospores, but is otherwise very similar. Arthur (2) has shown that this rust has its aecial stage on *Northoscordium bivalve* (L.) Britton, a species very similar and fairly closely related to *Ornithogalum umbellatum*. Although Arthur was not able to infect *Ornithogalum* with *Uromyces hordei*, the evident relationship of the two rusts invited further study. In March and April, 1920, a telial collection of *Uromyces hordei*, sent by R. S. Kirby from Norman, Oklahoma, was used four times in an effort to infect plants of *Northoscordium bivalve* and *Ornithogalum umbellatum*.

Abundant infection was obtained upon *Northoscordium* (Pl. 1, D) while no signs of infection were detected on *Ornithogalum*. Aeciospores from the infection on *Northoscordium* were sown two different times on seedlings of *Hordeum pusillum* and of common barley, *Hordeum vulgare*, which is susceptible to *Puccinia anomala*. Infection resulted only on *Hordeum pusillum*. Telia obtained from these cultures were overwintered and again sown upon *Northoscordium bivalve* and *Ornithogalum umbellatum* in March, 1921. Infection developed only on the *Northoscordium*. In addition, five different uredinial cultures of *Puccinia anomala* from different localities, while infecting barley varieties heavily, produced no infection when sown on *Hordeum pusillum*. It seems evident, therefore, that although *Uromyces hordei* and *Puccinia anomala* are somewhat closely related, they are distinctly different as to host specialization, in both the aecial and telial stages.

These results show that *Ornithogalum umbellatum* is an aecial host for the leaf rust of barley in this country as well as in Russia. The four widely separated localities from which the telial collections were made also would indicate that this is likely to be the case for this rust generally over the entire country. The writers have no evidence at the present time to show that the aecial stage is naturally produced, as aecia on this host have never been collected or reported in this country. However, it is entirely possible that they may be produced but have been overlooked, attention not having been directed to this plant, as the rôle of Star-of-Bethlehem as an aecial host was discovered quite recently and apparently is not generally known.

Star-of-Bethlehem may become, if it already is not, of considerable importance in barley-growing areas because of its tendency to escape from cultivation and become a weed. In some places, notably in the Southern States, this plant has become a pest almost equal in importance to wild onion (garlic), as far as occupying cultivated land is concerned. It is a bulbous plant, coming up in the early spring and dying down in midsummer. It multiplies rapidly by division of the bulb and may be scattered widely over the field in plowing and cultivating. It is obvious, therefore, that its presence in barley-growing areas is very undesirable.

Unlike the leaf rust of rye, the leaf rust of barley has not been shown to overwinter in the uredinial stage in this country. In consequence, the aecial host may be an important factor in the survival and spread of this rust. It must be acknowledged, however, that in the two places where the rust has been observed by us to be most severe, Arlington Experiment Farm, Rosslyn, Va., and Blacksburg, Va., Star-of-Bethlehem is not known as a weed.

## SUMMARY

1. The leaf rust of rye, *Puccinia dispersa*, is able to produce aecia on species of *Anchusa* in the United States.
2. *Anchusa officinalis*, and *A. capensis* are susceptible. *Nonnea rosea* may be infected occasionally with production of pycnia only. The other boraginaceous species tested remained uninfected.
3. *Anchusa capensis* has been found naturally infected by the leaf rust of rye and may become of some importance in the spread of the disease.
4. Apparently unlike the usual situation in Europe, the teliospores of the leaf rust of rye are capable of overwintering, and may germinate the following spring.
5. The leaf rust of barley, *Puccinia anomala*, from four widely separated localities in the United States has been used in inoculation experiments which have resulted in the development of aecia on *Ornithogalum umbellatum*, agreeing with results obtained by Tranzschel in Russia.

6. Although the aecial stage of *Puccinia anomala* has not been found occurring naturally, Star-of-Bethlehem possesses weed-like characteristics in this country which make it a dangerous plant near barley fields.

7. *Puccinia anomala* in its host specialization is distinct from the closely related *Uromyces hordei*. So far as known, neither is able to infect the hosts of the other.

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## PLATE 1

A.—Aecial stage of *Puccinia dispersa* upon leaf of *Anchusa officinalis*.

B.—Aecial stage of *Puccinia dispersa* upon leaf of *Anchusa capensis*.

C.—Aecial stage of *Puccinia anomala* upon leaves of *Ornithogalum umbellatum*, obtained from infection with teliospores from barley.

D.—Aecial stage of *Uromyces hordei* upon leaves of *Northoscordium bivalve*, obtained from infection with teliospores from *Hordeum pusillum*.



FALL SOWING AND DELAYED GERMINATION OF  
WESTERN WHITE PINE SEED <sup>1</sup>

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Experiments to determine the best time to sow seed of western white pine (*Pinus monticola*) have been under way in the northern Rocky Mountain region since 1912, partly in northern Idaho at the Priest River Forest Experiment Station, but mainly at the Savenac nursery on the Lolo National Forest in western Montana. Climate and soil combine to make the problem essentially local, but although the findings may not apply directly elsewhere the observations may be found suggestive.<sup>2</sup>

In order to understand the problem it is necessary to know something about what is termed the "hold-over characteristic" of western white pine seed. Like the other five-needled pines, this species has a strong tendency not to germinate all of its viable seed the first season after spring sowing. At Savenac nursery this delayed or second-season germination is often as high as 50 per cent, and has been anywhere from 4 to 91 per cent of the total number germinating from spring sowings. There are several theories regarding the cause of this habit. Chief among them are, first, the presence of an impermeable seed coat, and, second, the need of an after-ripening process in the embryo.

DISADVANTAGES OF SPRING SOWING AND HOLD-OVER  
GERMINATION

Table I is based on data secured from spring sowing during three different years and shows the proportion of hold-over germinations to have been high in every case. The figures give no reason to consider early spring sowing any better than late spring for western white pine; nor do seasonal variations in rainfall seem to be directly connected with the results obtained. Precipitation for the year 1914 was average, being but 0.5 inches above the mean amount, while 1915 and 1916 were both fairly moist with 2.27 and 3.59 inches, respectively, above the mean. Artificial sprinkling, of course, is employed to counteract the effect of too little rain in dry seasons.

TABLE I.—*Germination by seasons of spring-sown western white pine*

Time of sowing	Number of plots	Number of seeds	Average percentage of sown seed germinating			Average percentage of total germination which occurred during second season
			First season	Second season	Both seasons	
May 1, 1914.....	1	4,000	6.0	10.9	16.9	64.5
May 15, 1914.....	1	4,000	5.1	11.1	16.2	68.5
June 1, 1914.....	1	4,000	7.3	12.4	19.7	62.9
May 5, 1915.....	6	5,000	28.8	13.0	41.8	31.1
May 27, 1915.....	6	6,000	13.4	22.2	35.6	62.3
May 5, 1916.....	6	6,000	6.0	36.0	42.0	85.7

<sup>1</sup> Received for publication May 21, 1924—issued November, 1924.  
<sup>2</sup> The early experimental work in this project was carried on by E. C. Rogers, assisted by P. C. Kitchin. A number of their manuscript reports have been drawn upon to indicate the previous work.

It is very desirable to eliminate this hold-over germination for several reasons. The most obvious objection is that a very irregular stand of seedlings is produced. The seed-bed stand is uneven-aged, uneven in size, and uneven in its resistance to injury. Such irregular stands require heavy culling at the end of the second season or must be kept through the third season with its additional costs for all cultural operations. Either course is undesirable and expensive.

Together with hold-over germination we have to deal with a first-season germination from spring sowings which extends its activity throughout the summer. This aggravates the difficulties just mentioned, particularly the poor resistance. Seedlings originating late in the season are young and tender, and so undergo heavy losses. Death from the damping-off fungi is confined to seedlings three weeks old or less. Young seedlings are scorched by the hot sun of July and August and die more easily from slight drying of the surface soil than do their companions which are a month or two older. This necessitates the heavy expense of shading the seed-beds with lath frames. Then often 20 to 25 per cent of these weak, not yet lignified individuals are lost over winter. Frost heaves the ground in late fall or early spring when the soil is moist and exposed, and the weaker seedlings are heaved out. To avoid this trouble mulching the beds with straw or similar material is necessary, but this in turn results in the breaking of tender stems while applying and removing mulch.

In the endeavor to obviate these difficulties many attempts have been made to hasten the germination of the western white pine seed, among which may be mentioned: Stratification, sowing fresh seed, soaking in hot or cold water or in acids of different concentrations, dry and moist freezing, sowing in hotbeds, sowing of pregerminated seed, and mechanical methods of cracking, piercing, abrading or removing the seed coat. Most of these, however, are dangerously artificial methods which, experience has shown, tend to lower germinative capacity and at the same time do not uniformly accelerate germination.<sup>3</sup>

#### FALL- VERSUS SPRING-SOWING EXPERIMENTS

Because the results of these trials of spring sowing were all quite unsatisfactory, the possibility of fall sowing was considered. If the hold-over germination could thus be avoided the disadvantage of exposing newly made seed-beds for a longer time to the ravages of bird and rodent enemies, and to frost heaving of seed would be compensated for or could be controlled by other means. In actual practice the losses due to these causes have been small.

During the growing seasons from 1914 to 1916 at the Priest River Forest Experiment Station, observations concerning the best season to sow were taken from plots sown for other studies. In one instance, fall-sown plots had completed 83 per cent of their total germination before the end of May, while less than 14 per cent of the germination in spring-sown plots was complete at that time. Again fall sowings were 17 per cent higher in survival, and produced larger stock in every respect.

In the fall of 1913 and spring of 1914 at Savenac nursery six plots, each containing 2,000 counted seeds, were sown on different dates. Germination from fall-sown plots was complete about 15 days before that from spring-sown plots started. (See fig. 1.)

Twelve plots sown on October 27 and November 4, 1915, and on May 5, 1916, were watched during 1916. Hold-over germinations were much more numerous from spring sowing. Subsequent work has constantly given similar results. (See fig. 2 and 3.)

<sup>3</sup> A full discussion of this subject is given in a manuscript thesis presented in 1917 to the faculty of the Graduate School of Cornell University: ROGERS, E. C. DELAYED GERMINATION AMONG THE FIVE-NEEDLE PINES.

IMPORTANCE OF DATE OF FALL SOWING

The superiority of fall over spring sowing of this species was so marked in the early experiments that it seemed as if the problem were solved, but it soon appeared that the correct time in the fall season for doing the sowing was also very important.

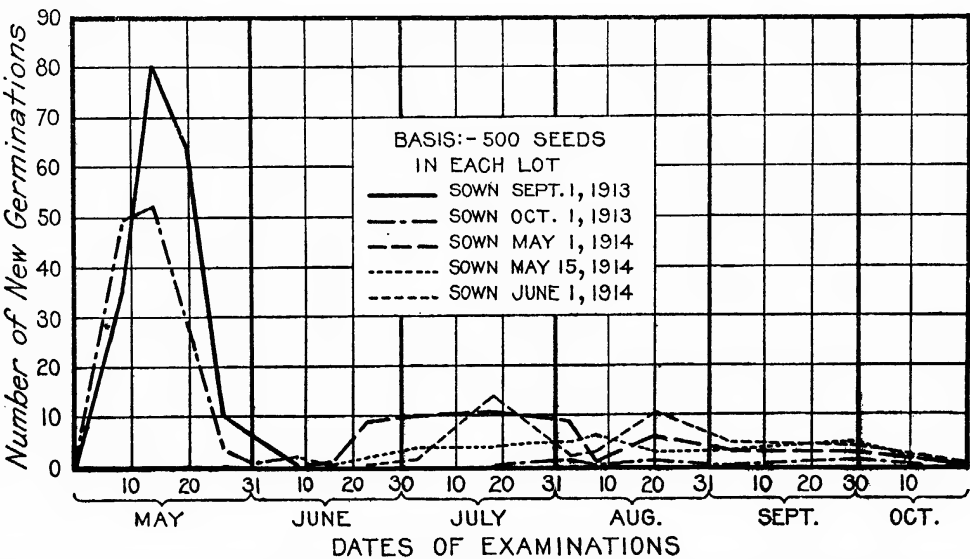


FIG. 1.—Germinative energy of western white-pine seed at Savenac Nursery as influenced by season of sowing

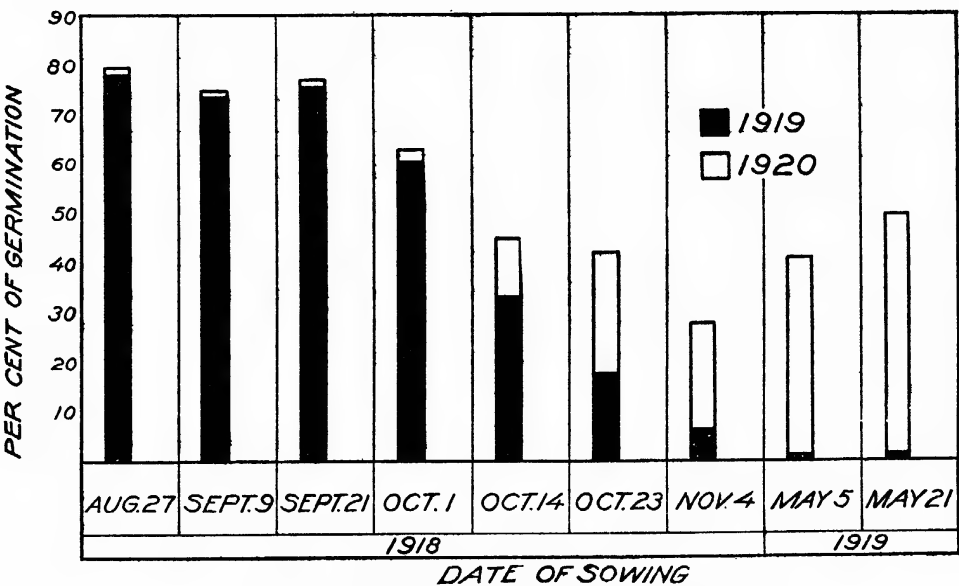


FIG. 2.—Results of sowing of Kaniksu seed, 1917

In the fall of 1916, at 10-day intervals from August 21 to October 28, twenty-eight plots of 1,000 seeds each were sown. From the results of this series early fall sowing is indicated as best, in order to reduce to a minimum the germination occurring after the month of June; but a different seed lot, germinating the highest total number, yielded only a small number of germinations after June 30, even from late sowings. Based on the highest number of seedlings germinating promptly the first season, September 1 was the optimum time to sow in 1916.



In the fall of 1917 sowings were made on September 7, 17, 27; October 6, 20, and November 3, with two lots of seed; 24,000 seeds were sown in samples of 1,000. These series showed no startling differences in September sowings, but the October sowings were inferior in promptness of germination and total numbers appearing the first season.

During the fall of 1918, fourteen samples of 1,000 seeds each were sown on various dates, utilizing seed from two sources. The first three weeks in September appear to have been the proper time to sow that year. The degree of promptness of germination was quite satisfactory for all of the first four sowings (August 27, September 9 and 21, and October 1), germination being practically all confined to the month of May. The graphs (fig. 2 and 3) show the total germinations and the proportion of hold-over. No seedlings were lost from "premature" germination; or, in other words, no seeds germinated during the fall of sowing

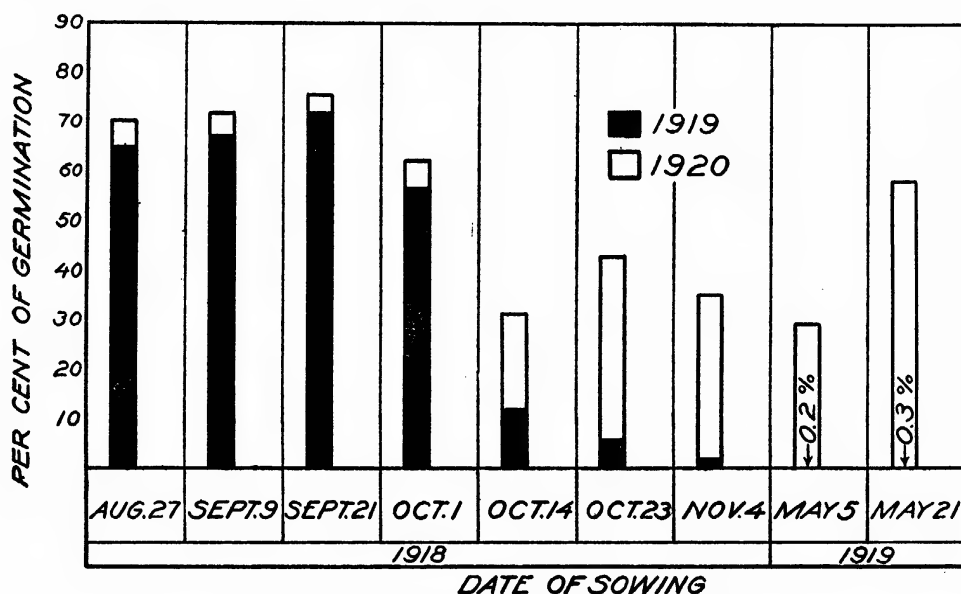


FIG. 3.—Results of sowing of Washington seed, 1917

only to succumb during winter. Except for the factor of germinative energy, or seasonal promptness, the graphs illustrate the essential points to consider in this problem, and appear to be typical of what may ordinarily be expected. The results of spring sowing on two different dates are also shown for the sake of comparison.

### SUMMARY OF ADVANTAGES OF FALL SOWING

Fall sowing of western white pine results in prompt and complete germination the following spring. Thus a regular and full stand of seedlings is secured. The heavy culling otherwise necessary, or its alternative of keeping the stock another year, is avoided. No shade or mulch at all is necessary, hence the entire cost of these three operations is saved. Handling the lath shade-frames was formerly a large item, because they had to be removed and replaced several times a season for weeding operations. A cost of \$66 for straw and \$181.50 for the frames and handling them is now saved each year at the Savenac Nursery as a result of these experiments. In short, it appears that we have avoided completely all of the disadvantages of spring sowings and hold-over germinations.

Figure 4 indicates that the best time in the fall for sowing western white pine seed at the Savenac nursery is during the first two weeks in September. According to the curve, the last few days in August may be as good, but this is not

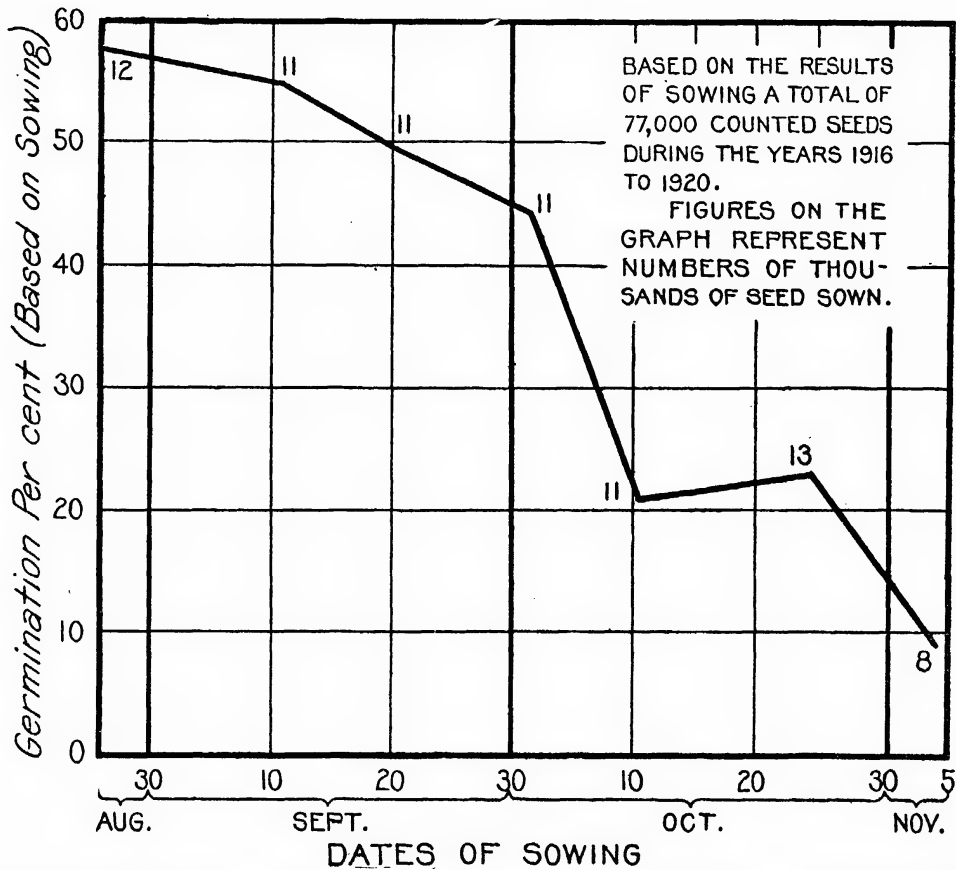


FIG. 4.—Germination of western white pine seed at Savenac nursery as influenced by time of sowing in the fall

certain. Trial sowings in August have not been numerous enough to fix the direction of the curve at that end. Furthermore, August sowings are subject to premature germination; that is, germination during the fall of sowing; and seedlings germinating prematurely do not survive the winter.



# THE EUROPEAN HARE (*LEPUS EUROPAEUS* PALLAS) IN NORTH AMERICA<sup>1</sup>

By JAMES SILVER

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Complaints of serious injury to young fruit trees in the Hudson River Valley of New York by European hares (*Lepus europaeus* Pallas),<sup>2</sup> locally known as jack-rabbits, led to an investigation of the situation by the Bureau of Biological Survey, United States Department of Agriculture, beginning in 1916. The writer was first detailed on this work in the fall of that year and at that time gathered considerable information relative to the introduction, habits, distribution, abundance, and economic status of these animals. A second visit to the infested area was made in January, 1918, to develop means of control. In January, 1922, letters of inquiry were sent to State and Federal game officials to collect further information regarding the spread of these hares and their effect on horticulture. The facts gathered leave no doubt that these animals are firmly established on this side of the Atlantic and that they are steadily increasing in numbers and gradually widening their range.

## HABITS

The European hares are very similar in habits to our western jack rabbits. Except for the well-known scream of fear and a faint cry sometimes used by the doe in calling her young, they are remarkably silent.<sup>3</sup> They seldom make use of burrows in the ground or of other places of concealment, but rely for protection either on escaping notice by remaining motionless, or, if detected, on their speed. They spend the day in slight depressions, or forms, only partially concealed in a clump of grass or in weeds or brush, but always in a location where their view or hearing is not cut off and from which they can make a quick "get-away." Once started they avoid thickets and make off in the open, depending upon their speed and dodging ability, almost entirely, to escape dogs, although sometimes they are credited with resorting to various tricks to accomplish this.

Their speed exceeds that of an ordinary dog, although some hounds can easily overtake them, and their habit of keeping to the open affords opportunity for observing their rate of travel. Near Lagrange, N. Y., two men reported on numerous tests which they made of this rate of speed whenever they "jumped" hares on the road in front of their automobile. Pressing a hare to its top speed along the road and with an eye on the speedometer they found that the hare traveled at an average rate of 30 to 33 miles per hour. In like manner they found that their collie dog was capable of making close to 30 miles per hour but could never summon the extra burst of speed necessary to catch the hare.

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<sup>1</sup> Received for publication Apr. 16, 1924—issued November, 1924.

<sup>2</sup> The European hare resembles closely the black-tailed jack rabbits of the western United States, having the characteristic long ears, much longer than the head, with a black area on the upper surface near the tips, and a conspicuous black area on the upper surface of the tail. It is much larger than our black-tail, an average adult weighing from 8 to 10 pounds or more, and reports of their weighing as much as 15 pounds are on record. The general color of the pelage on the upper-parts is a grizzled, buffy brown; the under-parts are white except on the breast, which is a decided reddish buff.

<sup>3</sup> MILLAIS, J. G. THE MAMMALS OF GREAT BRITAIN AND IRELAND. 3 v., illus. London. 1904-6.

The hare is apparently a solitary animal, more than one seldom being seen at a time, except perhaps in the breeding season. The breeding season, however, is indefinite, as young are said to be found at any time from early in spring till late in fall. There is uncertainty as to the number of litters brought forth each year, but it seems probable that there are at least two, with an average of two to three young to the litter. Barrett-Hamilton states,<sup>4</sup> that the fact that young may be found throughout the year makes it unlikely that there can, as a rule, be less than two or three litters. He further states:

Most observers have met with small numbers of leverets together up to, at most, four; but larger litters of five, six, seven, or more have occasionally been reported.

He also quotes J. C. Mansel-Pleydell as authority for the statement that, while five were once found together, three is the usual number with vigorous mothers; but with old does only two, and with young mothers one. The period of gestation is probably about 30 days, although definite information on the subject is not available.

### HISTORY OF INTRODUCTION

European hares were introduced into America solely as game animals for sport in coursing. They were at first liberated in New York State in a game preserve but were eventually turned loose in the open, as the preserve proved unsuited for coursing, even had the 9-mile fence that was erected been capable of retaining the animals.

There appear to have been five or more successful importations of these hares into America. Probably the most successful, from the standpoint of the subsequent rate of spread and increase, took place about 1893 at Millbrook, Dutchess County, N. Y. In that year a wealthy resident brought in several hundred from Hungary through a New York importing company, under State permit. This importation was the first of a number of his shipments, some of which consisted of 500 animals each, made at intervals of four or five years, the last being in 1910 or 1911. The number of hares introduced was increased by a smaller number imported and liberated on a neighboring estate. The high mortality during transportation made the expense of introduction very great, amounting to about \$10 a head.

An earlier importation seems to have been made at Jobstown, N. J., in 1888, but without the impetus of numbers given at Millbrook, N. Y. This importation is reported to have consisted of the brown hare from England (*Lepus europaeus occidentalis* de Winton). An importation is also reported at Bethlehem, Pa., and another at White Plains, N. Y. Information is lacking concerning the source of the hares liberated at these places. An account of the spread of European hares in Ontario, Canada, after being introduced on an estate near Brantford, Ont., has also been received.

### DISTRIBUTION AND ABUNDANCE

During the 30 or more years that have elapsed since the European hare first appeared in this country, the increase and spread has been slow but steady. The accompanying map (fig. 1) shows the distribution of the species in the United States as far as reported. The present range might be roughly described as extending from southern Vermont to central New Jersey and eastward 20 or 30 miles into Connecticut and Massachusetts, and to a limited extent westward across the Hudson and Delaware Rivers into extreme eastern Pennsylvania. Reports that are considered reliable have been used in mapping this distribution, but in some cases specimens are not available.

<sup>4</sup> BARRETT-HAMILTON, G. E. H. A HISTORY OF BRITISH MAMMALS. 1: 285-289. London. 1912.

The most notable spread of the hares has been eastward into Massachusetts and Connecticut. One report states that hares have recently been seen as far east as Stratford, Conn. Another report has them in the vicinity of Stafford, in north central Connecticut, but as there is doubt as to the identity of the animals, this record is omitted from the map. In 1915 a hare was shot as far north as Fair Haven, Vt., by the State Fish and Game Commissioner, but as no others have since been seen this far north, the Massachusetts-Vermont line may be considered the northern limit at present (1924).

A very few hares are reported in southern New Jersey, in Salem and Cumberland Counties. Although the report is not sufficiently definite for mapping, it

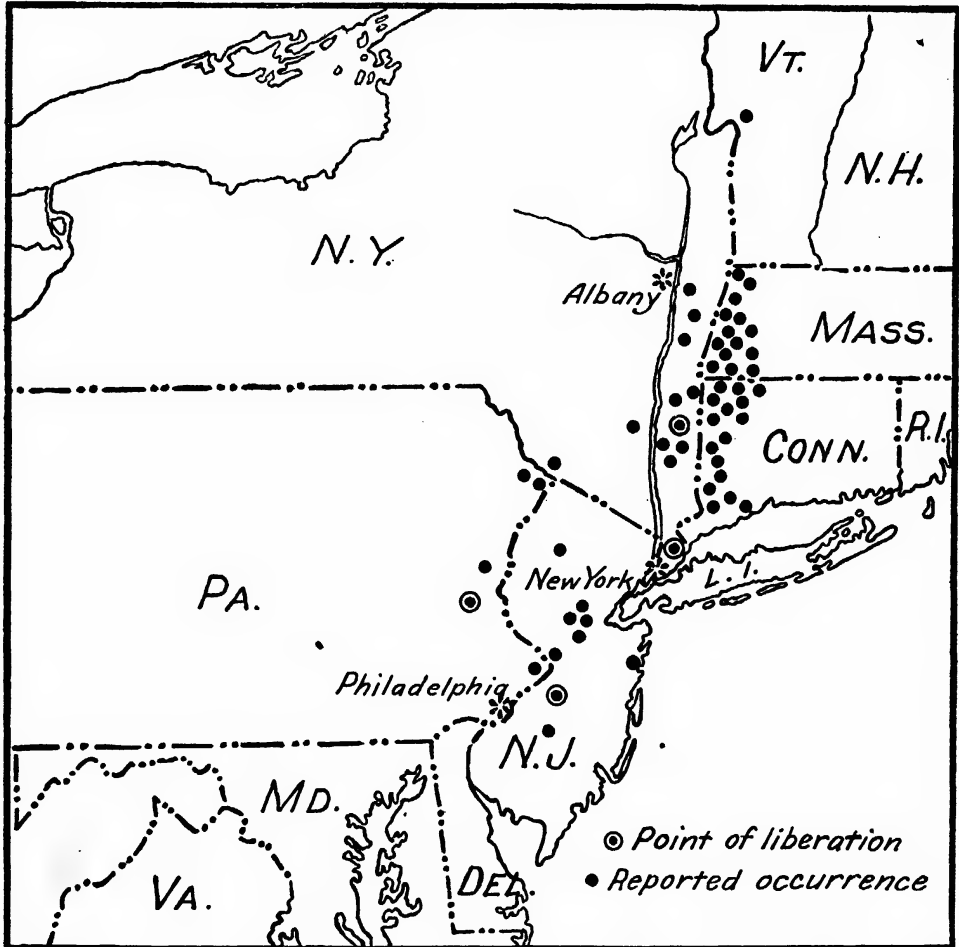


FIG. 1.—Distribution of the introduced European hare (*Lepus europaeus* Pallas) in the United States.

may be accepted, for, according to reliable information, the hares have been working south from the vicinity of Jobstown in recent years.

Samuel N. Rhoads, in 1903, reported upon the distribution of this hare in New Jersey as follows:<sup>5</sup>

This animal, escaped from domestication, has now become scattered over many localities in the northern half of New Jersey, especially in the parts radiating from Columbus. \* \* \* It is now found sparingly in the wild parts of Camden and Burlington Counties, N. J., and is regularly hunted in Bucks County, Pa., during the game season.

<sup>5</sup> RHOADS, S. N. THE MAMMALS OF PENNSYLVANIA AND NEW JERSEY. 266 p., illus. Philadelphia. 1903.

Definite information is lacking as to the extent of infestation in eastern Pennsylvania, although it is certain that hares are scarce there at present. Records are lacking also of occurrences west of the Hudson in New York. A few hares have been reported in the vicinity of Port Jervis, and the animals are reported quite common across the river from Poughkeepsie.

Prof. A. Brooker Klugh, of Queen's University, Canada, writes that the European hares are increasing and spreading rapidly in Ontario and that they occupy some 4,500 square miles adjoining Lake Erie on the north. Information concerning the species is being collected by interested Canadians with a view to publishing a report there on the matter.

The areas of greatest abundance of hares at present are eastern Dutchess County, N. Y., western Litchfield and Fairfield Counties, Conn., and southern Berkshire County, Mass. The hares in New Jersey do not seem to have increased so rapidly as in New York, but they are reported to be numerous in the vicinity of Stelton, Middlesex County.

The abundance of the hares is partially indicated by the number of bounties paid on them by Dutchess County from 1912 to 1917. Bounties paid for the first four years are as follows: For 1912, on 1,987 hares; 1913, on 1,879; 1914, on 3,054; and 1915, on 2,942. The figures for 1916 and 1917 are not available, but slightly over 3,000 bounties in each of these years must have been paid, inasmuch as approximately \$4,000 was spent in the six years in paying bounty at 25 cents per head. If the bounty was paid on half of the hares in the county each year, it can be estimated that there are 5,000 hares in Dutchess County, or an average of 6 hares to the square mile. The continued increase of hares, however, in spite of this drain upon their numbers, would probably indicate a much larger population. Based on a study of tracks in the snow near Sheffield, Mass., a population of 20 to 40 hares per square mile has been reported in that vicinity.

### DEPREDATIONS

The only serious complaint made against the European hare is the injury it causes to young orchard trees. This occurs only during severe winter weather when the natural food of the hare is not readily obtainable. Under such conditions, however, one hare can do great damage in a young orchard in a short time, and the presence of the animals in a fruit section is a constant menace unless suitable provision is made to protect the young trees.

The first noteworthy depredations of these hares occurred during the winter of 1900, when several young orchards near Millbrook were severely damaged. At this time an unsuccessful attempt was made to collect damages from the person who liberated the animals. The injury by hares was particularly severe during the winters 1909-10, 1912-13, 1915-16, 1917-18, and 1920-21, the unusually deep and persistent snows of these years forcing the animals to depend for subsistence upon the bark of trees and shrubs. The investigation following the winter of 1915-16 showed that a large proportion of the young orchards of Dutchess County had sustained losses from hare depredations. Many orchardists counted trees destroyed by the hundred, a number which often represented entire orchards.

The succulent bark of the younger trees is preferred by the hares, although no tree with smooth bark is immune from their attack. Every tree in one orchard of 200 large 5-year-old apple trees was badly damaged, and it was estimated that the losses in Dutchess County alone during the winter of 1915-16 exceeded \$100,000.

The hare shows a decided preference for apple trees. In one large orchard of apple, peach, and cherry trees at Lagrange, N. Y., several hundred apple trees were destroyed, while not one of the peach or cherry trees was touched. Shade trees, ornamental shrubbery, small fruit bushes, and the like are damaged to some extent, but such losses are relatively inconsequential.

The fact that a bounty was placed on the hares indicates that the people of Dutchess County considered them a pest. The county agricultural agent a few years ago voiced this opinion when he stated that hares were the worst pest the orchardists of the county had to contend with, as they were the only one that could not be successfully controlled.

### CONTROL MEASURES

The excellence of hares as game, together with freedom from State protection, will do much to lessen the danger of their ever becoming a serious pest, particularly in the more thickly settled orchard sections. Because of the great injury that a few hares can do in a single night, however, it is necessary that orchardists be urged to anticipate an attack and that they be instructed in methods of control. Poisoning and trapping are unsatisfactory, probably because of the difficulty in attracting these wide-ranging animals to a particular bait. In addition to shooting, methods of control include the use of woven-wire fences, individual tree protectors, repellent washes, and winter feeding.

Mechanical protectors often fail at a critical time because of drifting snows; repellent washes deteriorate rapidly; and winter feeding does not always accomplish its purpose. Any one of the three methods, however, will considerably reduce injury in case of attack.

Poultry netting 30 inches high, of 1-inch mesh, encircling the tree, is among the best of the mechanical protectors. Stakes or spreaders should be used to prevent the hares from pressing the wire against the bark and injuring it through the meshes. Various forms of wooden, paper, and cloth protectors are commonly used. They should be removed each spring, however, as they furnish retreats for insect pests when left permanently on the trees.

Of the repellent washes the concentrated commercial lime-sulphur wash is the most promising. Its adhesive qualities may be increased somewhat by the addition of half a pound of cheap glue to a gallon of the lime-sulphur sludge. This is applied to the trunk of the tree with a paint brush. During a severe winter two or possibly three applications may be necessary.

Feeding rabbits in winter to prevent their attack on the trees is practiced on the theory that it is cheaper to feed than to fight them. The most common practice is to leave the winter prunings of the trees scattered about the orchard.

### VALUE FOR FOOD AND SPORT

Hares are highly prized as food in Europe and, although in this country opinion is divided as to their palatableness, they find a ready market in the towns and cities throughout their range. Some who are familiar with both European hares and jack rabbits state that the European hare is far superior as food.

A minimum estimate of the present numbers of these hares in this country would be 50,000, which would represent nearly half a million pounds of meat. The meat value of those killed goes far toward offsetting the injury inflicted to orchard trees in the restricted area in which these hares are now found.

European hares are not protected (1924) at any season in New York, Connecticut, or in Berkshire County, Mass.<sup>6</sup> The farmers of Berkshire County made bitter complaint against them to the State Legislature, with the result that all protection was removed in this county. While there is no doubt that hares are a pest in sections where fruit is raised extensively, it is equally certain that they are an important asset as food and game. Their great speed and their characteristic habit of taking to the open when pursued make them a prime favorite in coursing, while they furnish excellent sport to thousands of individual hunters, as was contemplated in their introduction.

<sup>6</sup> LAWYER, G. A., and EARNSHAW, F. L. GAME LAWS FOR THE SEASON 1924-25. U. S. Dept. Agr. Farmers' Bul. 1444, 48 p. 1924. Animal publications.





# GROWTH ON CUT-OVER AND VIRGIN WESTERN YELLOW PINE LANDS IN CENTRAL IDAHO<sup>1</sup>

By C. F. KORSTIAN, *Associate Silviculturist, Appalachian Forest Experiment Station, Forest Service*

## PURPOSE OF STUDY

The western yellow pine (*Pinus ponderosa*), because of the superiority of its lumber, its abundance, and its accessibility, is the most important commercial timber tree in the Boise, Payette, Weiser, Idaho, and Salmon National Forests in central Idaho. It is of the utmost importance, therefore, that the stands of this species be so managed as to insure an adequate stand of reproduction on cut-over lands and to maintain at its maximum the potential productivity of the type. In order to secure reliable information upon the establishment of a new forest, its growth and development, and to determine the basic principles governing silvicultural practice in this type, the Forest Service established a series of 16 permanent sample plots on the Payette National Forest in central Idaho in 1913 and 1914. The results obtained through the remeasurement of these plots in 1918 and 1919 brought out many points of interest concerning the management of western yellow pine stands in this region which are well worth attention.

## GENERAL CONDITIONS ON THE PLOTS

Although these plots were established primarily to secure data on the growth of the reserved trees and natural reproduction in cut-over stands, unfortunately only three of them, plots Nos. 1-3, Table I, had been cut over when the logging operation was suspended in 1914. Nevertheless, they now afford a good comparison between the increment in cut-over and in virgin stands, showing behavior of advance reproduction in the latter. The cutting on these plots approached the method of clear cutting with scattered seed trees, although plot No. 1 had quite a number of trees left because they were too young. The uncut plots Nos. 4 to 16 give a good indication of the increment and mortality which may be expected in virgin stands. Furthermore, they afford interesting comparisons among three important sites common to the Intermountain region.

The sample plots were laid out in representative virgin stands under a variety of topographic and soil conditions.

The soil is a sandy to gravelly loam of granitic origin and is open, porous, and very gravelly on the south aspect. In the basins and on the north aspect the soil is less gravelly and more loamy because of a greater admixture of organic matter.

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<sup>1</sup> Received for publication Apr. 29, 1924—issued November, 1924.

TABLE I.—Number of living and dead trees, 4 inches diameter breast high and over, to the acre

Plot No.	Aspect	Species	Number of trees at first measurement	Increase or decrease (—) in number of trees	New trees entered	Number of dead trees	Volume of dead trees. <sup>a</sup>	
							<i>Cu. ft.</i>	<i>Per cent</i>
1	South	Western yellow pine	28.0	4.2	4.6	0.4	7.7	0.82
2	North	do	6.0	2.4	2.6	.2	11.0	4.66
3	Basin	do	7.8	6.0	6.0	.0	.0	.0
		Average (plots Nos. 1 to 3), cut-over.	13.9	4.2	4.4	.2	6.2	1.16
4	South	Western yellow pine	29.0	1.4	2.0	.6	122.9	2.74
		Douglas fir	3.6	.2	.2	.0	.0	.00
		Total	32.6	1.6	2.2	.6	122.9	2.31
5	do	Western yellow pine	36.6	5.2	6.0	.8	110.6	2.57
		Douglas fir	3.6	.0	.0	.0	.0	.00
		Total	40.2	5.2	6.0	.8	110.6	2.33
6	do	Western yellow pine	21.0	3.2	3.6	.4	94.8	2.86
		Douglas fir	2.6	— .2	.0	.2	10.8	5.76
		Total	23.6	3.0	3.6	.6	105.6	3.01
7	do	Western yellow pine	32.6	1.6	3.0	1.4	198.2	7.93
		Douglas fir	.8	.0	.0	.0	.0	.00
		Total	33.4	1.6	3.0	1.4	198.2	7.31
		Average (plots Nos. 4 to 7), virgin.	32.4	2.8	3.7	.9	134.3	3.30
8	Basin	Western yellow pine	27.4	1.2	1.4	.2	57.0	1.43
		Douglas fir	11.0	— .4	.0	.4	99.8	7.76
		Total	38.4	.8	1.4	.6	156.8	2.98
9	do	Western yellow pine	21.6	5.4	6.0	.6	78.8	2.81
		Douglas fir	19.4	— .4	.4	.8	26.7	1.45
		Total	41.0	5.0	6.4	1.4	105.5	2.27
10	do	Western yellow pine	26.4	— 1.8	.2	2.0	64.6	1.90
		Douglas fir	27.0	.4	.4	.0	.0	.00
		Total	53.4	— 1.4	.6	2.0	64.6	1.25
11	do	Western yellow pine	16.0	— 1.6	.8	2.4	238.9	8.71
		Douglas fir	43.4	.2	.4	.2	7.8	.20
		Total	59.4	— 1.4	1.2	2.6	246.7	3.73
		Average (plots Nos. 8 to 11), virgin.	48.0	.8	2.4	1.6	143.4	2.64
12	North	Western yellow pine	16.0	— .4	.2	.6	326.8	12.88
		Douglas fir	38.0	— 2.0	.4	2.4	148.4	6.20
		Total	54.0	— 2.4	.6	3.0	475.2	9.63
13	do	Western yellow pine	15.8	3.8	4.6	.8	70.4	3.69
		Douglas fir	109.4	4.8	9.2	4.4	36.4	.90
		Total	125.2	8.6	13.8	5.2	106.8	1.80
14	do	Western yellow pine	22.8	— .4	.6	1.0	70.2	2.44
		Douglas fir	30.6	2.8	3.8	1.0	47.3	4.24
		Total	53.4	2.4	4.4	2.0	117.5	2.94
15	do	Western yellow pine	6.2	1.8	1.8	.0	.0	.00
		Douglas fir	57.2	4.2	5.8	1.6	63.3	2.36
		Total	63.4	6.0	7.6	1.6	63.3	1.71
		Average (plots 12 to 15), virgin.	74.0	3.6	6.6	3.0	190.7	4.11
16	do	Western yellow pine	24.2	2.9	3.6	.7	81.5	3.19
		Average (plots Nos. 4 to 16), <sup>b</sup> virgin:						
		Western yellow pine	23.0	1.9	2.8	.9	110.0	3.82
		Douglas fir	21.7	.6	1.3	.7	27.5	2.13
		Total	44.7	2.5	4.1	1.6	137.5	3.30

<sup>a</sup> This includes only those trees which died since the plots were established.<sup>b</sup> Averages weighted by plot areas for total area of 80 acres.

Like the bulk of virgin western yellow pine forests, the uncut stands are made up mostly of mature or overmature even-aged groups. Some immature groups have, however, become established in the openings in the forest cover caused by the death of old trees. Each of the plots is 5 acres in extent, with the exception of plot No. 16, which covers 20 acres. Plots Nos. 4 to 15 are located on Big Pine Creek, at an altitude of 4,000 to 4,500 feet. Plots Nos. 1 to 3 are on Carpentier Creek, at an elevation of approximately 3,700 feet, while plot No. 16 is located on Poorman Creek, at about the same altitude as plots Nos. 1 to 3. All of these plots are in pure stands of western yellow pine. Of the uncut plots, Nos. 4, 5, 6, and 7 are on ridge tops and south aspects where western yellow pine occurs in practically pure stands. Plots Nos. 8, 9, 10, and 11 are in basins and coves where both species are found in mixture, but with western yellow pine still in the ascendancy, while plots Nos. 12, 13, 14, and 15 are located on north aspects, with Douglas fir (*Pseudotsuga taxifolia*) predominating, but with an admixture of yellow pine.

The conditions in the cut and uncut plots as to vigor, mortality, and growth during the five-year period will be brought out in summarizing the results of remeasurement.

### INCREASE IN NUMBER OF TREES

The stocking on the cut-over plots is conspicuously incomplete, while the characteristic occurrence of western yellow pine in groups gives an appearance of still greater inadequacy. There are indeed many small saplings on the ground, established before the cutting took place, but these have not yet had time to grow into the 4-inch class, and so did not appear in the records used in this study. The cut-over plots show a somewhat greater increase in number of trees than the virgin plots; an average increase of 4.2 trees an acre during the five-year period as compared with an average increase of 2.5 trees on the uncut plots (see Table I). This difference will in all probability become more pronounced in the course of the next decade or two. It can reasonably be expected that there will be comparatively little loss due to disease and overmaturity in cut-over areas if all defective trees and those of poor vigor be removed at the time of cutting.

### INCREASE IN VOLUME

Table II is a summarized statement of the increment on the sample plots during the five-year period. In order to show the actual amount of new wood added each year by growth, the gross increment is also given. Since loss of volume through death of trees can not be avoided under present economic conditions, which render impracticable a return in less than 40 years and possibly longer, it is plain that the figures on net growth, indicating the actual gain or loss in volume, are at present the more significant. It is important to note that without cutting, many of the causes of loss in these overmature stands can not be eliminated.

The table following reveals a wide range in the net increments on the various plots. Other things being equal, the growth on basin plots Nos. 8 to 11 should have been the highest of all, but because of a large number of young thrifty trees (especially Douglas fir) on the northern aspects the growth is actually greater on plots Nos. 13 to 15. The low increment on plot No. 12 is due largely to the high percentage of insect-infested trees. The next lowest rate of net growth per cent is on plot No. 11, where *Dendroctonus* bark-beetle infestation also is serious. Mistletoe, which is prevalent on plot No. 9, does not appear to retard the growth of Douglas fir so much as do bark beetles. Net losses are shown for only three of the uncut plots (plots Nos. 10, 11, 12), though the western yellow pine shows a net loss on five plots in all and Douglas fir on two.

TABLE II.—Increment per acre of trees 4 inches diameter breast high and over

Plot No.	Aspect	Species	Volume per acre at first measurement <sup>a</sup>		Periodic annual increment per acre <sup>b</sup>			Percentage of periodic annual cubic foot increment	
					Gross	Net		Gross	Net
			<i>Cu. ft.</i>	<i>Board ft.</i>	<i>Cu. ft.</i>	<i>Cu. ft.</i>	<i>Bd. ft.</i>		
1	South	Western yellow pine	942.0	4,780	46.8	45.3	285	5.0	4.8
2	North	do	235.9	1,228	15.0	12.8	75	6.4	5.4
3	Basin	do	424.6	2,352	26.6	26.6	164	6.3	6.3
		Average, plots 1 to 3, cut over.	534.2	2,787	29.5	28.2	175	5.5	5.3
4	South	Western yellow pine	4,483.1	28,584	32.1	7.5	54	.7	.2
		Douglas fir	839.6	4,410	10.7	10.7	63	1.3	1.3
		Total	5,322.7	32,994	42.8	18.2	117	.8	.3
5	do	Western yellow pine	4,306.8	27,212	46.2	24.1	161	1.1	.6
		Douglas fir	437.0	1,902	5.7	5.7	31	1.3	1.3
		Total	4,743.8	29,114	51.9	29.8	192	1.1	.6
6	do	Western yellow pine	3,316.1	21,752	27.0	8.0	37	.8	.2
		Douglas fir	187.5	782	3.8	1.6	9	2.0	.9
		Total	3,503.6	22,534	30.8	9.6	46	.9	.3
7	do	Western yellow pine	2,499.3	14,992	38.2	-1.4	0	1.5	-.1
		Douglas fir	212.8	1,010	2.5	2.5	12	1.2	1.2
		Total	2,712.1	16,002	40.7	1.1	12	1.5	.04
		Average, plots 4 to 7, virgin stands, south aspect.	4,070.6	25,161	41.6	14.7	92	1.0	.4
8	Basin	Western yellow pine	3,978.6	25,454	25.2	10.9	82	.6	.3
		Douglas fir	1,285.6	5,626	18.8	-6.1	-25	1.5	-.5
		Total	5,264.2	31,080	44.0	4.8	57	.8	.1
9	do	Western yellow pine	2,805.5	18,032	17.9	-1.8	-25	.6	-.1
		Douglas fir	1,838.1	8,040	26.7	20.0	96	1.5	1.1
		Total	4,643.6	26,072	44.6	18.2	71	1.0	.4
10	do	Western yellow pine	3,400.7	21,830	19.3	6.4	68	.6	.2
		Douglas fir	1,757.0	7,008	34.4	34.4	164	2.0	2.0
		Total	5,157.7	28,838	53.7	40.8	232	1.0	.8
11	do	Western yellow pine	2,741.4	18,044	9.0	-50.7	-308	.3	-1.8
		Douglas fir	3,880.1	17,034	43.2	41.2	180	1.1	1.1
		Total	6,621.5	35,078	52.2	-9.5	-128	.8	-.1
		Average, plots 8 to 11, virgin stands, basin.	5,421.8	30,267	48.6	13.6	58	.9	.3
12	North	Western yellow pine	2,537.4	16,640	15.5	-66.2	-476	.6	-2.6
		Douglas fir	2,395.0	9,540	17.9	-19.2	-70	.7	-.8
		Total	4,932.4	26,180	33.4	-85.4	-546	.7	-1.7
13	do	Western yellow pine	1,909.4	12,298	12.3	-1.8	-6	.6	-.1
		Douglas fir	4,033.3	15,560	74.9	67.6	270	1.9	1.7
		Total	5,942.7	27,858	87.2	65.8	264	1.5	1.1
14	do	Western yellow pine	2,877.4	19,754	43.7	29.7	170	1.5	1.0
		Douglas fir	1,114.6	4,070	23.0	13.5	52	2.1	1.2
		Total	3,992.0	23,824	66.7	43.2	222	1.7	1.1
15	do	Western yellow pine	1,017.3	6,578	14.4	14.4	102	1.4	1.4
		Douglas fir	2,684.2	10,350	52.0	39.3	156	1.9	1.5
		Total	3,701.5	16,928	66.4	53.7	258	1.8	1.5
		Average, plots 12 to 15, virgin stands, north aspect.	4,642.2	23,698	63.4	19.3	50	1.4	.4
16	do	Western yellow pine	2,556.2	15,964	68.3	52.0	355	2.7	2.0
		Average, plots 4 to 16, virgin stands: <sup>c</sup>							
		Western yellow pine	2,881.1	18,439	35.9	11.7	80	1.2	.4
		Douglas fir	1,291.6	5,333	19.6	13.2	59	1.5	1.0
		Total	4,172.7	23,772	55.5	24.9	139	1.3	.6

<sup>a</sup> Cubic volume includes the solid contents of the entire stem, including the stump, of all trees 4 inches and over at breast height. Board foot volume includes all trees 12 inches and over at breast height, volumes read from a Scribner decimal C volume table for the Payette Forest.

<sup>b</sup> Plots Nos. 1 to 7, inclusive, Nos. 10, and 13 to 16, inclusive, show increment for a 5-year period, while plots Nos. 8, 9, 11, and 12 show it for a 4-year period.

<sup>c</sup> Averages are weighted by plot areas for total area of 80 acres.

A striking difference exists between the rate of growth in the cut-over and the uncut stands. The cubic volume of the cut-over stands has increased at the rate of 5.3 per cent per annum since the plots were established, while the virgin stands show an annual increase of only 0.6 per cent for the same period. With an indicated annual increment of 24.9 cubic feet per acre it is apparent that the loss—by windfall, bark beetles, mistletoe, or otherwise—of one large tree containing 249 cubic feet would completely offset 10 years' growth on 1 acre of virgin timber.

The actual average loss was found to be 6.2 cubic feet an acre for the five-year period in the cut-over stands and 137.5 cubic feet an acre in the virgin stands during the same time. This comparison is gratifying, although the situation still demands improvement of silvicultural practice. It is evident that through a careful selection of trees to be left standing on cut-over areas the maintenance of a desirable rate of volume growth will be made possible.

The percentage of trees showing lack of vigor, as emphasized by Table III, is a good criterion of the general condition of the stands. In this study slight injuries were not considered; the tree had to be decidedly lacking in vigor before being so classified. For example, Douglas fir which showed light mistletoe infection or western yellow pine with slight porcupine injury, if otherwise thrifty, were still classified as vigorous. Since a general lack of vigor and thrift usually precedes death, this will be treated along with mortality.

TABLE III.—Defective trees on sample plots in 1918 and 1919, with summary of important causes of lack of vigor, in percentages of total living trees and cubic volume at beginning of period

Cause of lack of vigor	Cut-over plots Nos. 1 to 3		Virgin plots Nos. 4 to 16					
	Western yellow pine		Western yellow pine		Douglas fir		Total	
	Trees	Cubic volume	Trees	Cubic volume	Trees	Cubic volume	Trees	Cubic volume
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Mistletoe infection.....					3.37	4.66	1.59	1.47
Suppression.....	2.94	0.57	1.65	0.11	2.35	.16	2.01	.13
Porcupine injury.....			.65	.14			.34	.10
Defective tops <sup>a</sup> .....	.37	.01	2.81	3.96	.90	1.24	1.91	3.07
Unclassified.....			1.70	1.10	1.80	2.37	1.75	1.44
Total, all causes.....	3.31	.58	6.81	5.31	8.42	8.43	7.60	6.21

<sup>a</sup> This group includes such conditions as tops broken in logging, spike tops, and stag-headed trees.

CAUSES OF LOSS

Table IV is a record of mortality from different causes. Of the agents listed as responsible for the death of the trees, the most important are bark beetles, windfall, and mistletoe. In many cases large mature and overmature trees were killed, resulting in a large volume loss.

TABLE IV.—Causes of death of trees 4 inches diameter breast high, and over, in percentages of total living trees and cubic volume at the beginning of period <sup>a</sup>

Cause of death	Cut-over plots Nos. 1 to 3		Virgin plots Nos. 4 to 16					
	Western yellow pine		Western yellow pine		Douglas fir		Total	
	Trees	Cubic volume	Trees	Cubic volume	Trees	Cubic volume	Trees	Cubic volume
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Windfall	-----	-----	0.44	9.65	0.75	0.63	0.59	0.65
Insects	-----	-----	2.49	2.85	.46	.97	1.50	2.27
Mistletoe	-----	-----			.29	.27	.14	.08
Suppression	-----	-----	.39	.01	.35	.02	.37	.02
Logging	-----	-----			-----	-----	-----	-----
Porcupines	0.48	0.09	.11	.001	-----	-----	.06	.002
Unclassified	.96	1.07	.33	.29	1.33	.24	.82	.28
Total	1.44	1.16	3.76	3.811	3.18	2.13	3.48	3.302

<sup>a</sup> This includes only those trees which died since the plots were established.

A maximum loss of 12.87 per cent in the five-year period was found on one uncut plot. Although this loss was occasioned by the death of three large over-mature trees, it is nevertheless alarming. Table IV shows that insect attacks, particularly those of the western pine beetle (*Dendroctonus brevicomis*), are responsible for a volume loss over twice as great as that caused by all other agents, and that this has been confined to the virgin stands and to the killing of individuals or small groups of trees. Under normal conditions the infestations are often sporadic, threatening for a year or two, and then decreasing. However, while no serious epidemics have occurred in recent years in this locality, it must not be forgotten that this insect is very destructive to western yellow pine throughout the West and constitutes a real menace which, from time to time, assumes very serious proportions, possibly aggravated by the advent of favorable climatic conditions. Practically all the insect injury noted occurred in trees above the sapling stage, and sufficient insect activity has been shown in virgin stands to warrant foresters giving it careful attention.

On these plots windfall was confined to the mature and overmature trees in the larger diameter classes. On the national forest timber sales this is being partially controlled through the selection of wind-firm individuals for seed trees and through the removal of very tall trees on exposed situations after adjoining trees which protect them are cut. As a matter of fact, wind-throw here was not nearly as serious as it appears to have been in the Whitman National Forest in eastern Oregon (11).<sup>2</sup> It is gratifying to note that no wind-throw occurred on the cut-over plots. Since a large proportion of the windfall occurs within four or five years after cutting and the remainder in rapidly decreasing amounts, the exercise of reasonable precaution in marking will undoubtedly eliminate the danger of heavy loss.

Western yellow pine in the vicinity of these sample plots is not infected with mistletoe (*Razoumofskya campylopoda*), although on the basaltic soils of the western division of the Payette National Forest and the nearby Weiser National Forest heavy infections occur. There is a suggestion here of an interesting causal relation between soil type and the local distribution of mistletoe, although the significance and constancy have not been determined. Douglas fir is frequently infected with mistletoe (*Razoumofskya douglasii*). Indeed, on plot No. 8

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 1148.

this infection assumed rather serious proportions, causing a loss of 3.09 per cent of the volume. While the loss due to mistletoe is not alarmingly high on any of the plots, the indications are that the death rate will increase from year to year. The information at hand indicates that the cutting of infected trees is the most practical method of control (6). Every effort should therefore be made to remove moderately and heavily infected trees when cuttings are made.

Death from suppression obviously results in a relatively small volume loss, although the number of dead trees may be large. The habitual occurrence of western yellow pine in fairly open stands which would seem to predicate a small amount of suppression is, to some extent, counterbalanced by the tendency to form scattered groups, with resultant heavy losses in the smaller diameter classes. The later records on these plots will supply valuable information as to the relative light requirements of the two species, their resistance to overtopping and, on the cut-over plots, their ability to recover upon being released.

It is noteworthy that on the cut-over plots all of the general lack of vigor and thrift is either the result of suppression which took place before the cutting and from which the western yellow pine had not recovered, or is due to the loss of a considerable part of the crown through careless felling of adjacent trees during logging. It would appear that for trees showing a lack of vigor on plots Nos. 1 to 3 inclusive, 3.31 is too high a percentage for recent cuttings, even though the trees comprise only 0.58 per cent of the total cubic volume on the cut-over area. (See Table III.) The need for greater care in logging to reduce the injury to reserved trees and to utilize all suppressed trees which are merchantable can scarcely be overemphasized. The plots show a significantly high percentage of trees in the mature and overmature virgin stands lacking in vigor and consisting of injured, defective, and decadent trees.

Porcupines have caused some injury in certain localities. The actual volume loss due to this agent is small. Saplings are frequently killed by girdling, but large trees are rarely killed. However, the leaders are often girdled, which sets the tree back from 5 to 15 feet or even more in height. New leaders are usually developed, but constrictions and pronounced crooks remain as evidence of the attack, which may even appreciably affect the amount and grade of lumber sawed from trees which recover.

### NATURAL REPRODUCTION

One of the main objects of silvicultural management is to secure prompt restocking after cutting. In fact, a forest in which restocking does not follow cutting is doomed ultimately to annihilation. In the forests of the Intermountain region, where artificial regeneration is subject to numerous adversities and an almost prohibitive cost (?), natural reproduction is of the utmost importance. Natural reproduction in the western yellow pine type, therefore, has been one of the foremost subjects of investigation in central Idaho. The more important results of these studies have already been published and therefore in the following paragraphs only the principal results will be summarized.

The effect of sheep grazing upon coniferous reproduction and the extent to which this damage can be reduced by more careful use of the range was made the subject of an intensive study by Sparhawk (10). Detailed information on germination, survival and causes of death of seedlings with particular reference to grazing injury was secured on 151 small sample plots in the Payette National Forest. This study emphasized the general principle that the extensive browsing of coniferous reproduction and the less palatable forage species is the best evidence that the range is being overgrazed. In many instances on cut-over areas in the course of regeneration it may be desirable to reduce materially or even to eliminate grazing, particularly that of sheep.



Adverse climatic conditions, while still important influential factors, do not so completely dominate the reproduction problem in central Idaho as they do in the Southwest (9). Yet it is considered desirable to retain a sufficient number of trees to preserve forest conditions, in addition to insuring an adequate seed supply, since a heavy opening of the stand increases the danger from wind, excessive evaporation, deficient moisture, and frost injury (3, 4, 5).

As the result of special studies (5) failure of reproduction was found to be due more to high mortality the first three years following germination than to a lack of germination. By far the greatest mortality was found in seedlings less than a year old. Very few over 6 inches high die in any one year. A leaf disease, caused by *Phacidium infestans*, has caused a significant loss among seedlings and saplings of the fairly tolerant Douglas fir and promises to continue as a cause of mortality on the plots. Another leaf fungus (*Hypoderma deformans*) was frequently found to be the cause of fairly heavy losses of western yellow pine seedlings and saplings and ranks along with suppression as a cause of mortality in this species on northern aspects and in basins, especially where the cover is rather dense.

The same field studies showed that the most serious causes of death, aside from the two diseases just mentioned, are in the order of their importance: Drought, browsing and trampling by grazing animals, especially sheep, girdling by rodents, winter-killing, including excessively low temperatures, frost injury, and rodents or birds which bite off the newly germinated seedlings. Seedlings and saplings 15 to 20 years old were occasionally found girdled by rodents. Balanced against this is the fact that rodents render considerable aid in disseminating and burying the seed, thereby promoting favorable conditions for satisfactory germination.

One of the most noteworthy results of these studies has been to emphasize the great importance of advance reproduction. The establishment of advance growth is a long slow process, doubtless requiring as much as 20 years or even more to secure adequate restocking on the poorer sites. Moderately heavy cuttings appear to be fully justified provided advance growth is reasonably abundant and provided healthy, vigorous seed trees are left as insurance against loss of the advance reproduction by fire. It is also evident that efforts to deliberately change the composition of the stand by the method of cutting will produce few tangible results. Western yellow pine is holding its own on potential yellow pine sites and it can be succeeded by Douglas fir only in the tension zone between the two types or in the Douglas fir type. A large number of Douglas fir seedlings can be found, but owing to a heavier mortality extending over a longer period of years, fewer seedlings actually become established than in the case of western yellow pine. Where the two species occur in mixture, as in the basins, the pine in the juvenile stage grows faster than Douglas fir. However, these different rates of juvenile growth do not continue beyond about 40 years of age when Douglas fir surpasses the pine in diameter growth at least.

#### APPLICATION OF RESULTS IN SILVICULTURAL PRACTICE

When the national forests of the West were placed under Government administration, little exact information on the characteristics and requirements of the important native forest trees was available upon which to base rules for silvicultural practice. As a result of repeated fires, grazing, and insect infestations, the forests were generally understocked and often had a preponderance of overmature and decadent timber and a deficiency of trees of the intermediate age classes from which to select vigorous, thrifty seed trees. All too often advance reproduction was poorly distributed or lacking. In addition there was no sale for defective timber.

Economic conditions have improved to some extent, but still only one cut will be possible for a long time. The marking rules must frequently be based on a compromise between economic necessity in logging practice and the best silvicultural practice.

The distribution of the reserved trees over the area is a matter of great importance. Except for the danger of wind-throw on exposed ridges, it is inadvisable to leave groups of trees of any considerable area untouched, since such blocks are inimical to both acceleration of growth and even distribution of new reproduction. Leaving trees in groups neutralizes the advantageous effects of cutting, and on the better sites may interfere with western yellow pine reproduction. Great care should be exercised in selecting trees for increased volume growth and enhanced quality of the subsequent cut. These conclusions have been substantiated by Dunning's studies (1, 2) in California. He has also clearly pointed out that the crown furnishes the most reliable criterion of what may be expected from reserved trees. A dense, bright-green, pointed crown is indicative of a thrifty tree. Trees with long narrow crowns are generally growing more rapidly than average trees of the same size. As a general principle, it is undesirable to leave mature or overmature seed trees because even on the best sites the death of one large tree will greatly reduce the net increment and the loss will be material of the highest quality. Furthermore, satisfactory growth can not be expected from trees of either western yellow pine or Douglas fir over 30 inches in diameter, even on the best sites in this region.

The marking practice in effect in the central Idaho forests is, in many respects, quite similar to that outlined by Munger (8) under the selection system or a modified form of it for the management of the western yellow pine type in eastern Oregon. The present marking practice in central Idaho provides for reserving a considerable nucleus of vigorous immature standards and a sufficient number of sound, thrifty mature trees to make up the necessary quota. These may be desired for the purpose of maintaining the continuity of the forest cover, for increased volume and value increment in a second cut, or to insure adequate seed for regenerating the stand. All defective, diseased, and suppressed trees should be marked for cutting unless needed as fire insurance or seed trees. No defective or diseased tree should be left standing if it is evident that it will not live until the next cutting, unless it is absolutely needed for silvicultural purposes. When it becomes necessary to reserve trees among the larger diameter classes, vigorous, thrifty trees of good form and development should be selected. It is thus evident that the marking on each individual area must be varied to meet the silvicultural requirements of the forest. The importance of careful, intelligent marking of timber on cutting areas can not be overemphasized, since this is the means by which rational silvicultural management is actually secured in practice.

### SUMMARY

This report presents the first important results of a growth study which is still incomplete. Permanent sample plots are used to compare cut-over and virgin forests as to condition of stands, mortality, and increment. The cut-over areas are in a much thriftier and more vigorous condition. In the virgin stands the loss of vigor is caused principally by bark-beetle infestations, mistletoe infection of Douglas fir, wind-throw, and suppression. These causes of loss were largely eliminated from the cut-over stands. Porcupines are responsible for a relatively small amount of injury.

The average rate of net volume growth in relation to the volume of the stand is strikingly greater in the cut-over than in the virgin stands. In the latter, the annual loss through death and decay practically nullifies the annual growth.

Great care should be taken in silvicultural markings to reserve, as seed trees, only thrifty individuals which can reasonably be expected to survive until the next cut is made and to continue to grow at a profitable rate. Every precaution should be taken to avoid injury to advance growth at the time of logging because of its supreme importance in regenerating the uneven-aged forests of western yellow pine promptly.

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## PLATE 1

A.—Cut-over stand of western yellow pine on a south-facing slope. The presence of saplings and small-sized trees gives the appearance of a "selection" cutting. The opening and the absence of a ground cover of shrubs is typical of ridges. Payette National Forest (plot 1).

B.—Heavy cutting on a north-facing slope. After six years reproduction is still deficient on this area (plot 2), a condition evidently due to the inability of the small-sized trees left after cutting to supply sufficient seed.





## PLATE 2

A.—The advance reproduction is deficient in this virgin stand of western yellow pine on a south-facing slope of moderate steepness. Ground cover is not dense on such sites and the lack of advance reproduction emphasizes the need for care in marking such stands, if prompt restocking is to be assured. Payette National Forest (plot 5).

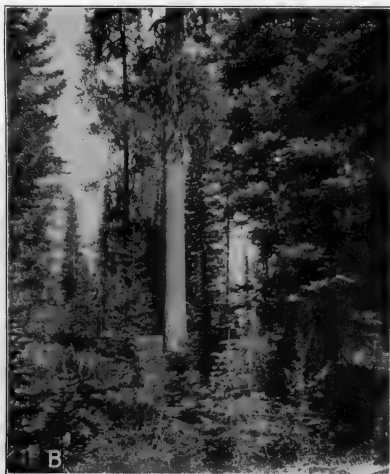
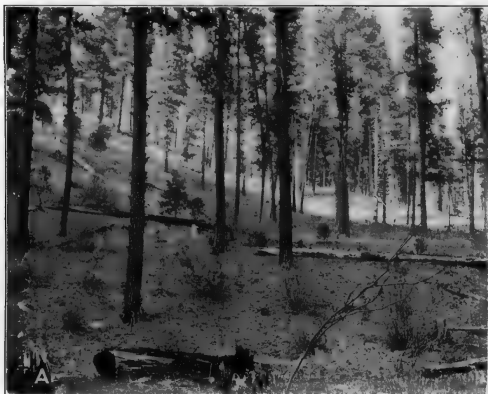
B.—A steep south slope necessitates light cutting as in the case of this mature stand of western yellow pine on a steep south slope (plot 6). On such situations, particularly near the tops of the ridges, cutting should be light to avoid heavy wind-throw and excessive drying of the soil. Payette National Forest.

### PLATE 3

A.—Large, thrifty trees must be reserved to reseed such areas. A mature stand of western yellow pine and Douglas fir typical of the basins in the Payette National Forest. Advance reproduction is deficient on this area (plot 8), except in the openings. When such stands are cut over, satisfactory restocking must depend upon reseeding by vigorous trees of large diameter left for that purpose.

B.—Abundant advance reproduction under a mature stand. Unusual care should be exercised in cutting such stands as this one of western yellow pine and Douglas fir to release and preserve the abundant young growth, particularly of yellow pine. Payette National Forest.





# SOME FACTORS AFFECTING REPRODUCTION AFTER LOGGING IN NORTHERN IDAHO<sup>1</sup>

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In the virgin forest of northern Idaho, natural reproduction of desirable forest tree species after cutting is often very scant and disappointing. Not only do the defective and prolific weed species of western hemlock and grand fir prevent good establishment of western white pine, but also good reproduction and establishment of the pine on exposed slopes and flats is in most cases very difficult to obtain. The latter difficulty comes about either through failure of the seed to germinate or because the seedlings, many of which may spring from seed already in the forest floor at the time of cutting, do not withstand the hot, dry summer weather and the drying out of the soil.

In order to throw light on this question of germination and survival, certain investigations, recorded in this paper, were made by the Priest River Forest Experiment Station in northern Idaho. These have to do with the effect of large clearings as compared with shelterwood and the uncut forest, germination of seed and establishment of seedlings on the various surfaces found after logging, and the influence of different aspects.

## INFLUENCE OF LARGE CLEARINGS

In the first place, the mere opening of the forest by cutting affects the physical conditions of the site in a very marked degree, both by increasing air and soil temperature, evaporation, and rate of transpiration, and by causing extremes or dangerous fluctuations which prove injurious to seedlings. In order to gain information on this point, studies were conducted during the dry and warm summer of 1919 on sites offering comparisons of three different conditions: (1) 250 feet within an old and uncut virgin forest of western white pine (*Pinus monticola*), western red cedar (*Thuja plicata*), western larch (*Larix occidentalis*), and grand fir (*Abies grandis*); (2) at a point 400 feet east of the first site under about one-third overhead cover provided by leaving defective hemlock (*Tsuga heterophylla*) and other species; and (3) on a wide and complete clearing to the south of the other two sites where all of the overhead trees were removed and the site fully exposed to sun and wind. These three locations were all found on a broad river bench at an elevation of 2,300 feet in the Priest River valley.

Each station was equipped with maximum and minimum air thermometers of the United States Weather Bureau pattern, and air thermographs were placed in the stations in the open and in the uncut forest. These instruments were placed 4½ feet above the ground within suitable shelter boxes. At each station there was one psychrometer and one Livingston cylindrical porous-cup atmometer. The latter was placed 6 inches above the ground, the bottle containing the water being entirely below the surface.

Daily soil temperature readings and semiweekly soil-moisture samples were obtained at a depth of 6 inches near each station on a bare plot of ground 1

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<sup>1</sup> Received for publication June 19, 1924—issued Nov., 1924.

meter square, away from all roots. The records, except those of soil moisture, were taken at 5 p. m. each day from July 12 to August 31. A summary of the data obtained is given in Tables I and II.

TABLE I.—*Physical conditions within the forest and on large clearings*

(Summaries of measurements July 12 to August 31, 1919)

Stand conditions	Air temperature		Relative humidity 5 p. m.	Daily evaporation	Soil temperature at depth of 6 inches	Soil moisture at depth of 6 inches
	Average maximum	Average minimum				
Open.....	°F. 87.7	°F. 39.5	Per cent 30.7	Cc. 35.3	°F. 63.2	Per cent a 31.6 b 36.7
One-third cover.....	85.3	44.3	33.0	26.9	59.9	a 23.5 b 28.1
Uncut.....	80.3	46.2	35.0	16.0	54.7	a 28.0 b 32.6

a July measurements.

b August measurements.

TABLE II.—*Daily march of air temperature in the forest and on large clearings*

(Thermograph records for August in degrees Fahrenheit)

Location	A. M.						P. M.					
	2	4	6	8	10	12	2	4	6	8	10	12
In open.....	42.7	40.4	39.5	50.4	64.5	76.7	82.4	83.3	78.5	61.2	51.0	46.1
In forest.....	50.7	47.9	46.0	47.6	57.7	67.4	74.1	76.1	73.6	65.9	56.6	53.9

The records in Table I show that the high points of air and soil temperatures in the open average from 7 to 8 degrees higher than within the uncut forest, and that the range in air temperature in the open is 48.2 degrees but only 34.1 degrees in the forest. These differences, accentuated by a lower humidity and greater evaporation, which naturally intensify the effect of drought on exposed surfaces, are factors which must be carefully weighed in providing suitable environments for seedling establishment of western white pine or other moisture-loving species. It should be stated that the slightly lesser soil moisture observed under the forest than on the open plot is considered due to root activity under the forest, for every part of the soil under the mature forest comes within reach of certain roots, whereas on the open plot where no trees exist, root competition is eliminated. In neither case, forest or open, did soil moisture conditions show any material difference or appear critical. The injurious effects of the exposure are expressed rather by greater evaporation and presumably much higher rates of transpiration, higher soil temperatures, or greater weed and grass competition on the open area than elsewhere.

There is other evidence that complete openings one-quarter mile or more in extent render the site very unfavorable to natural establishment of hemlock and cedar and Engelmann spruce (*Picea engelmanni*), and somewhat precarious for white pine seedlings. This is expressed by the disappearance in such locations of many of the typical moisture-loving plants which usually grow underneath and among the white pine stands, such as *Coptis trifolia*, *Cornus canadensis*, *Aralia nudicaulis*, *Asarum caudatum*, *Circaea pacifica*, *Claytonia perfoliata*, and *Galium boreale*; and the appearance of species less dependent upon soil

moisture and shelter such as *Rubus strigosus*, *Lonicera utahensis*, *Linnaea borealis americana*, *Spiraea lucida*, *Ceanothus velutinus*, *Salix scouleriana* and a great many grasses and sedges frequently found in the more open western yellow pine (*Pinus ponderosa*) and Douglas fir (*Pseudotsuga taxifolia*) stands.

This change in the physical condition of the site, brought about by clear cutting of the forest, has been observed by the writer in numerous instances over a period of years. Such clearings ordinarily show poorer natural restocking of western white pine and other moisture-loving species compared with north and east aspects; also relatively fewer seedlings in the presence of heavy sod and dense weeds than under partial shade or among lighter vegetation. On one such recently clear-cut flat the seedlings which germinated from seed in the duff were counted for three consecutive years. These showed a mortality of about 50 per cent, although they had no weed or grass competition. Numerous counts and observations, made on large clear-cut areas from eight to twelve years following cutting, show natural reproduction at a standstill except where sheltered by the border of the uncut timber. What takes the greatest toll of young seedlings and creates the most critical physical barrier to natural reproduction on large clearings and south and west aspects in northern Idaho is the fact that the prevailing and desiccating westerly winds in summer always strike the warm and sunny places. Naturally this hindrance, by exposure, drought, competition, and dense vegetation, becomes more acute and more dangerous to natural regeneration on sites with poor soil and on southerly exposures than on sites with deep, moist soil or on northerly aspects or in small sheltered spots such as result from partial cutting of the forest. The influence, therefore, of large clearings on flats and sunny slopes is often to defeat altogether the reestablishment of western white pine and other moisture-loving species.

### INFLUENCE OF SURFACES ON GERMINATION AND SURVIVAL

To test out the influences of the different kinds of surfaces such as ordinarily are found on a logged area, seven seed beds, each 4 feet square, were sown with western white pine seed in the fall of 1918. The tests embraced surfaces of ashes, fresh soil, charcoal and ashes, wood and duff charcoal, partly burned, and unburned surfaces. Each bed contained 2,000 fresh white pine seeds and was protected from birds and rodents by proper screens. The ash surfaces were the result of brush burning and the bare soil surface was prepared by raking off the needles, duff, and humus layer. In all cases the seed was sown without raking it in. The results are given in Table III.

TABLE III.—*Germination and survival of western white pine on different surfaces*  
(Sowing of 2,000 seed per plot in the fall of 1918)

Date of germination	Germination percentages by types of surface conditions						
	Un-burned deep duff	Partly burned deep duff	Un-burned duff and decayed wood	Mixture of charcoal and partly burned duff	Bare loose soil	Char-coal and ashes	Deep loose ashes
1919.....	0.2	4.9	0.3	0.6	12.7	10.2	17.7
1920.....	9.5	53.9	12.8	9.9	47.9	28.7	15.2
Total.....	9.7	58.8	13.1	10.5	60.6	38.9	32.9
Percentage of survival 1921.....	96.9	100.	53.4	36.5	48.2	62.5	95.8

The best germination took place on partly burned deep leaf duff and bare soil, and the burned surfaces in general showed better germination than the unburned plots. The fairly prompt germination on deep ashes is worth noting. This is the surface that gave greatest germination the first season. On the unburned

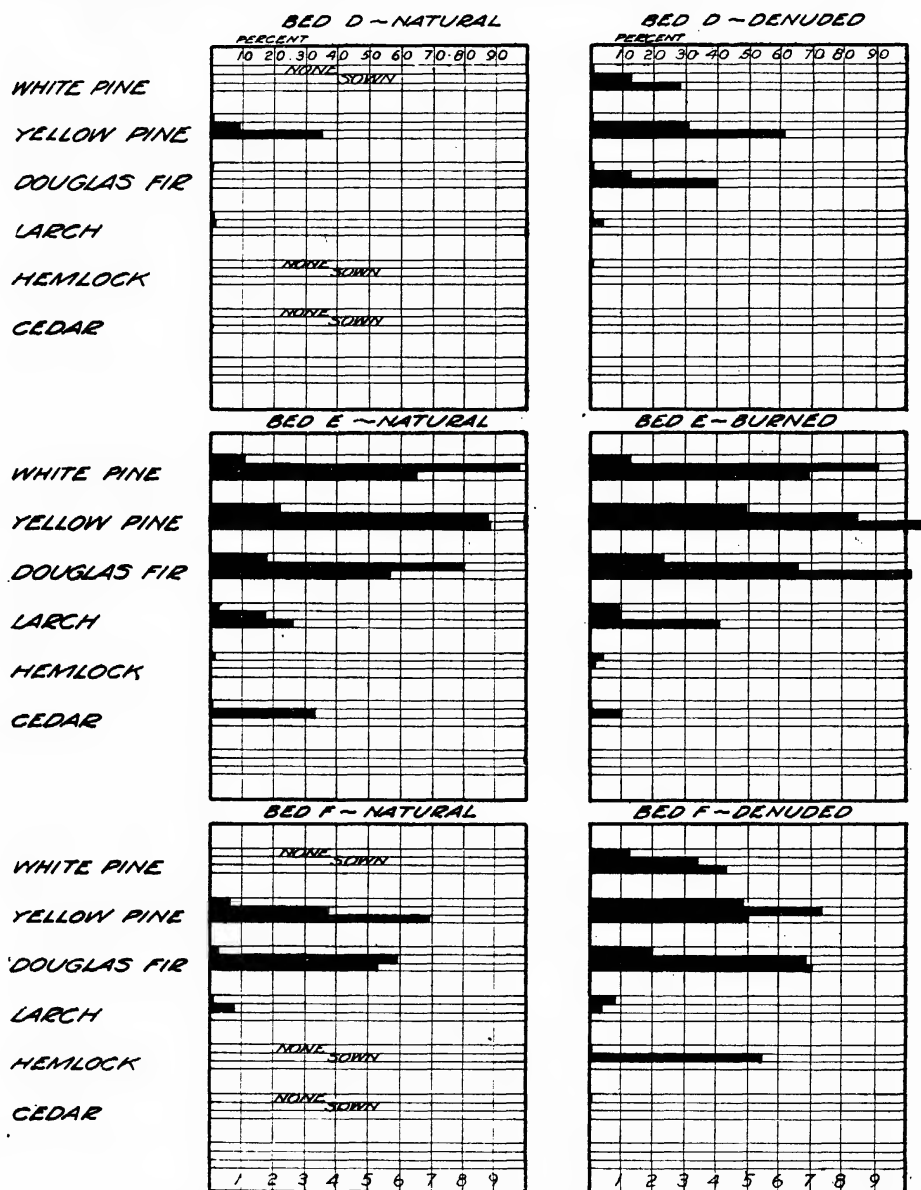


FIG. 1.—Some earlier results of germination and survival on different surfaces, by species. The three horizontal bars opposite each species indicate (1) germination, per cent of seed sown; (2) survival, spring of 1915, per cent of germination; (3) average height in inches, fall of 1917

surfaces the second year's germination, which is a marked characteristic of western white pine, was by far the strongest; in fact, practically all of it occurred during the second season. In the matter of survival the differences are less distinct, but, taking it all in all, the burned surfaces showed results fully as good as the unburned. It should be mentioned also that the seedlings on the ashes and the bare soil were taller and sturdier than those on unburned ground, and that the survival of the trees germinating on the burned surface showed an exceptionally high percentage.

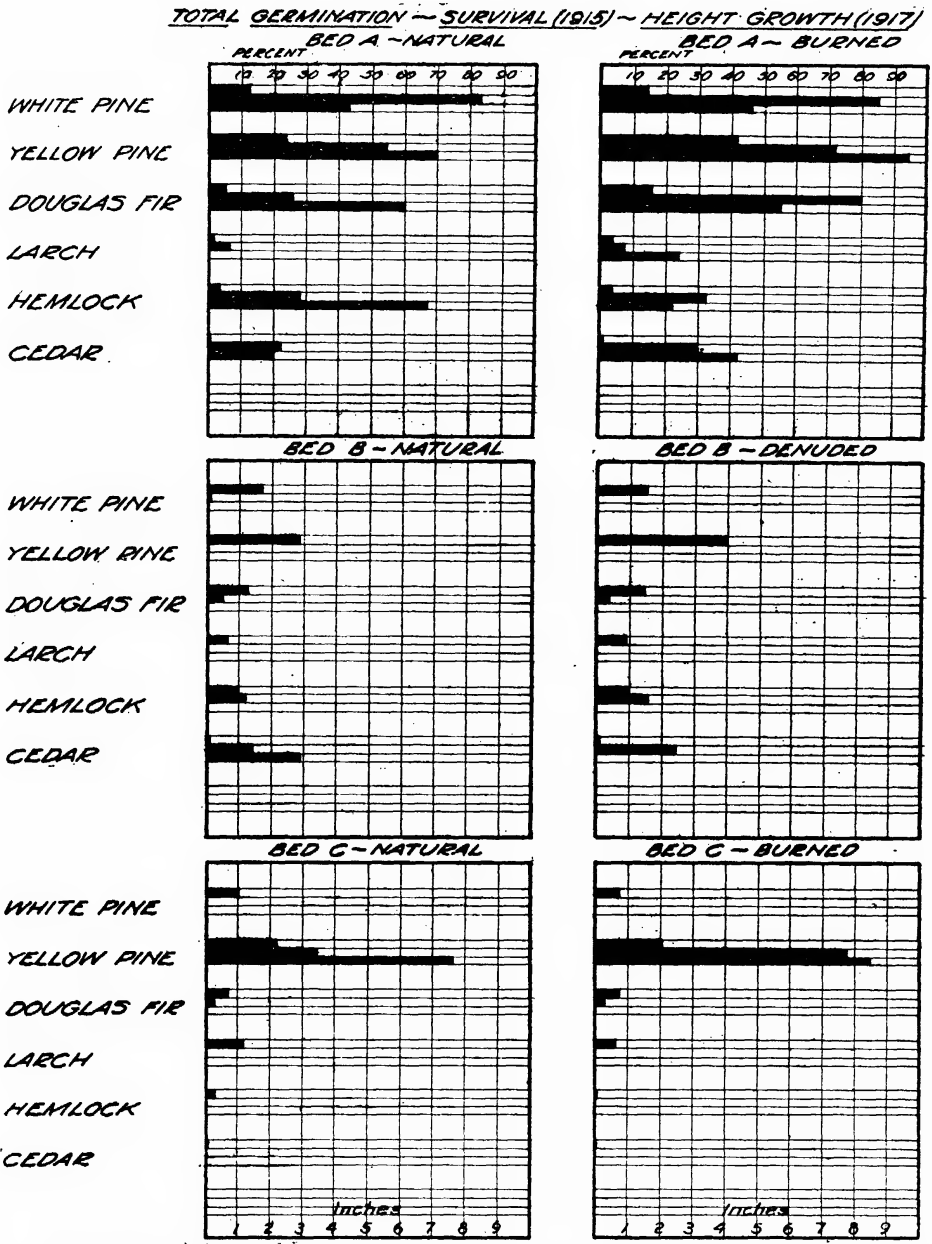


FIG. 2.—Some earlier results of germination and survival on different surfaces, by species. The three horizontal bars opposite each species indicate (1) germination, per cent of seed sown; (2) survival, spring of 1915, per cent of germination; (3) average height in inches, fall of 1917

Some of the earlier results are shown in figures 1 and 2.

Satisfactory germination of these species took place both on the bare, loose mineral soil and on the ashes, and the survival on both was good. The duff surfaces show poor results for all three species and more distinctly so here than in the 1918 series.

Even in the absence of exact measurements of moisture content and surface temperature, one would surmise that the great fluctuations in moisture taking place on duff surfaces would be a disadvantage to seed germination. It is of interest to record a few of the more outstanding and characteristic results of a great many tests of duff moisture contents and fluctuations made at the Priest River Forest Experiment Station by H. T. Gisborne and the author, dating back to 1913.

In the fall of 1920 a second test was made by sowing on three different surfaces ashes, duff, and bare loose soil. At this time two more species (western larch and Engelmann spruce) were added which were also sown on bare soil, ashes, and duff surfaces. Separate beds 4 feet square were used for the pine and long beds with three divisions of surface within one bed for spruce and larch. Germination and survival counts were continued for two years, giving the following results:

TABLE IV.—*Germination and survival on different surfaces, by species*

(Sowing of 2,000 seed per plot in the fall of 1920)

Surfaces	Western white pine		Western larch		Engelmann spruce	
	Total germination	Survival of germination	Total germination	Survival of germination	Total germination	Survival of germination
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Ashes.....	24.1	94.0	20.0	36.0	24.0	64.0
Duff.....	1.6	58.0	4.3	35.0	14.0	0.0
Bare soil.....	24.5	a 13.0	13.4	64.0	35.0	67.0

a Seedlings were affected by a fungous disease.

## FLUCTUATIONS IN MOISTURE AND TEMPERATURE OF DUFF COMPARED TO BARE SOIL

Moisture fluctuations in duff are seasonal, periodic, and diurnal. The seasonal fluctuation is expressed by a gradual loss which progresses with the advance of the summer toward a minimum reached early in July. The periodic fluctuation involves a drop after each rainfall in summer with a minimum moisture content of 10 per cent or lower within 8 to 10 days after the rain, depending upon the amount of rain and the drying conditions (fig. 3). There is also a diurnal fluctuation which reaches the maximum in early morning and the minimum in late afternoon. Tests for 19 consecutive clear days during 1923 showed minima at 5 p. m. from 10 to 14 per cent, and maxima at 8 a. m. from 20 to 24 per cent.

TABLE V.—*Temperature fluctuations in duff and on bare soil*

(Average of daily thermograph records in degrees Fahrenheit June 1 to 30, 1921)

Type of soil	A. M.						P. M.					
	2	4	6	8	10	12	2	4	6	8	10	12
Duff.....	55.8	54.9	53.5	54.3	56.8	61.2	66.1	65.0	63.3	61.1	58.8	56.8
Bare soil.....	51.4	50.1	50.4	53.8	60.9	63.1	70.3	65.5	62.1	58.4	55.3	53.2

Naturally, the duff dries out to a greater depth than ash or bare soil surfaces so that seedlings with short and tender roots, like spruce, hemlock and cedar, are often overtaken by drought before the roots become properly established in the underlying soil. In soil and ash surfaces saturation may be as great as in duff but drying out is much less rapid. In this case the entire underlying soil must become dry before surface moisture gives out. Again, moisture adheres closely to the seeds when sowed in ashes or on soil, in that these become imbedded in, or coated with, soil or ashes and are at all times in close contact with the surface. From the standpoint of moisture, therefore, soil and ash surfaces present more favorable seed beds than duff or loose litter.

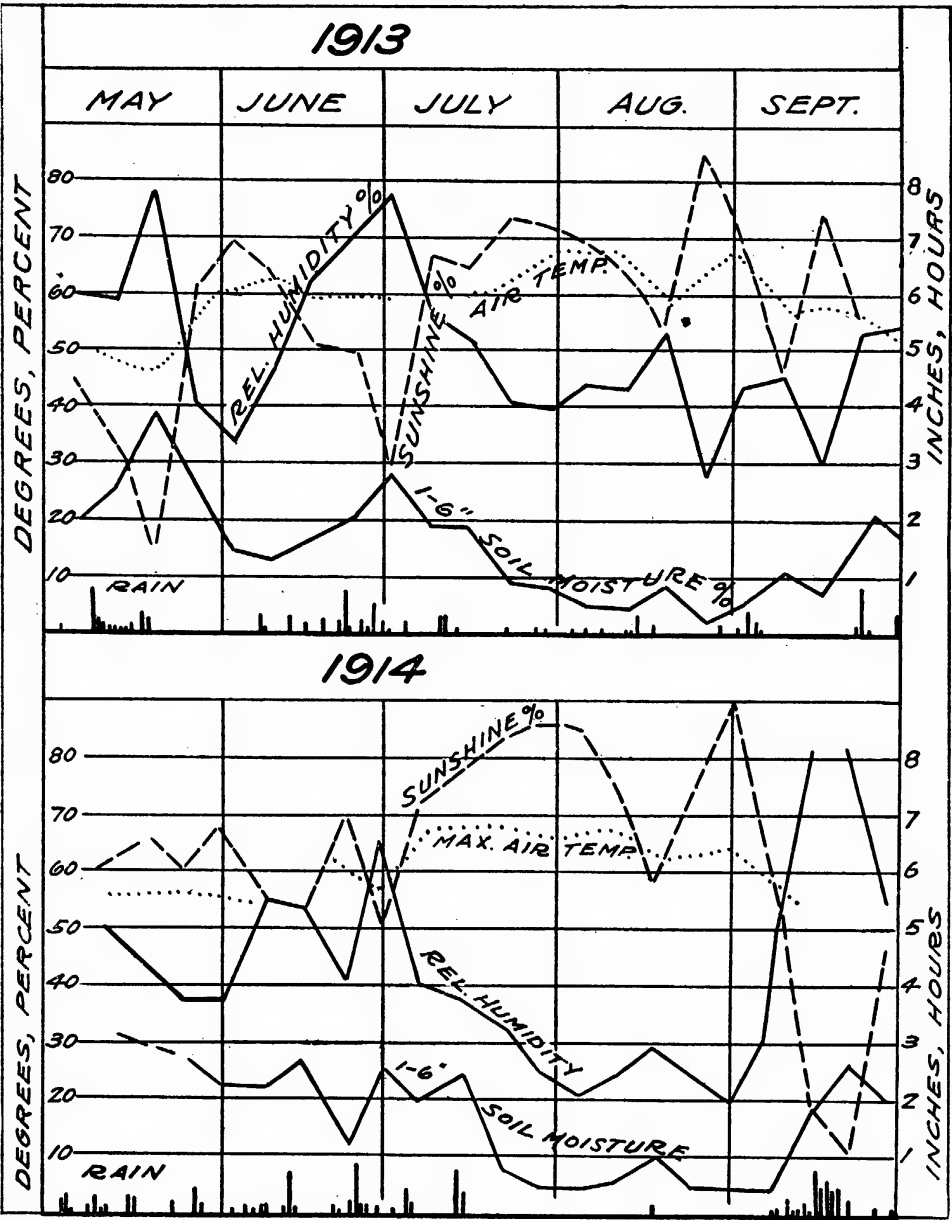


FIG. 3.—Correlation of relative humidity and soil moisture, Priest River Experiment Station

But since moisture alone does not induce germination, the variations in temperature must also be tested. With this in view, soil thermographs were used in the 1920 series, one bulb being placed directly under the surface of the bare soil and another similarly placed under a duff surface. Continuous records were obtained from April 15 to June 30, 1921. This covers the germination period. Results from June 1 to 30, the period of most active seed germination, are given in Table V.

Between 9 a. m. and 3 p. m. from June 1 to 30, the soil surfaces average from 2° to 5° F. higher temperature than the duff. These are averages for clear and cloudy days alike; during sunny days there were differences from 7° to 10°.

This higher temperature on soil surfaces is naturally explained by the lower specific heat of soil, 0.2, as compared with duff, 0.3. The greater heating during the day is a condition which greatly stimulates germination on bare soil and ash surfaces over that of duff and litter. Soil and ash surfaces are, therefore, more



favorable than duff or litter to early germination of seed both from the standpoint of moisture and temperature conditions.

Fortunately, during the period of seed germination, the great extremes of surface soil temperature, which, if the soil were dry, would prove injurious to the tender seedlings, are effectively held in check by the high water content in soil and ash surfaces, for water has a very high specific heat of 1.0.

INFLUENCE OF ASPECT

Later in the summer, during July and August, the bare soil may become altogether too hot or too dry for the tender seedlings and cause death by wilting

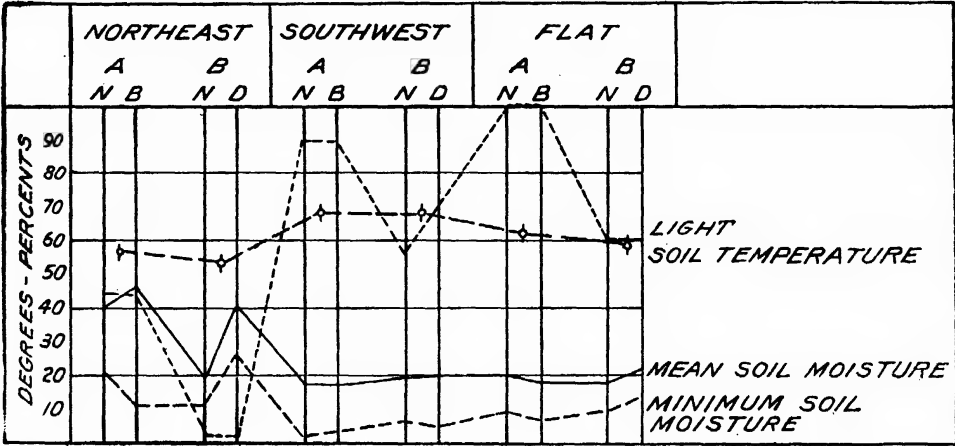


FIG. 4.—Season average of soil temperatures, 1913. N, natural surfaces; B, burned surfaces, D, denuded surfaces

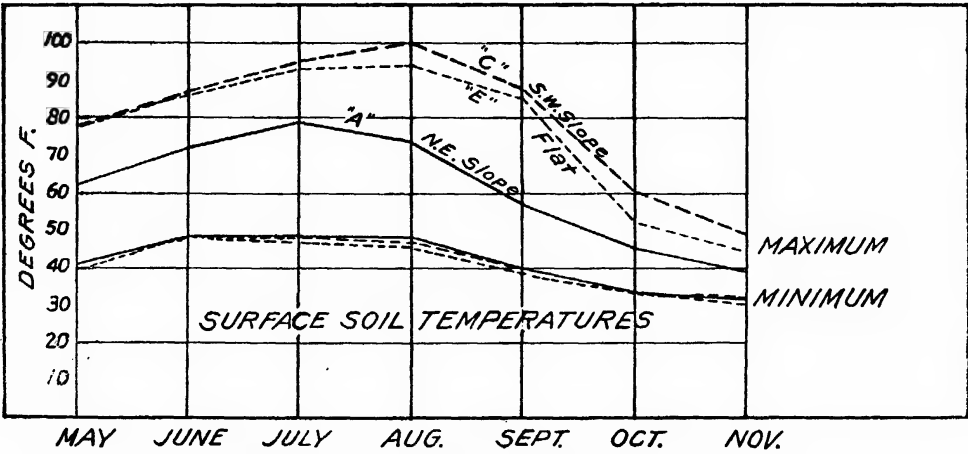


FIG. 5.—Season average, 1913, beds A, C, and E

or burning of the stems, or for want of sufficient soil moisture. It is from this standpoint that aspect and the degree of slope play an important part on seedling survival. The figures in Table VI merely summarize the high points of data obtained over a period of five years, from 1911 to 1916, on a northeast slope, a southwest slope, and a flat, all within one-half section of land. (See also fig. 4.)

From the data in Table VI, and as shown in figure 5, it is seen that the absolute maximum surface temperatures and moisture content on the northerly slope never become critical for seedlings. But on the exposed flats and on southerly aspects the maximum surface temperatures have registered from 115° to 125° F., and the soil moisture has been as low as 3.4 to 0.5 per cent. These extremes cause death both by direct contact and by excessive evaporation, for on such

very hot days the relative humidity of the air reaches the extremely low points of 10 to 20 per cent. From the many sowing tests installed in 1913 on the northeast slope, the southeast slope, and the flat with sandy soil, in the course of which 84 different samples of seed were used, germination of western white pine, cedar, hemlock, larch, and grand fir was proved to be as good on exposed sites as on the most favored aspects; but only western yellow pine and Douglas fir seedlings survived the peak of the hot weather on the southwest aspect.<sup>2</sup> The greater death rate occurred when soil became very warm and dry. Often sites such as result from unrestricted clearings on south and west aspects, therefore, become too dry and too hot for seedling survival of western white pine and other moisture-loving species.

TABLE VI.—*Extremes of surface soil temperature and moisture on different aspects in August*

(Records of 1913, 1915, and 1916, northern Idaho)

Location	Surface temperature		Surface soil moisture		Soil moisture at 6-12 inches depth	
	Average	Maximum	Average	Minimum	Average	Minimum
	° F.	° F.	Per cent	Per cent	Per cent	Per cent
Northeast slope.....	60.2	85.0	43.6	10.7	42.9	11.9
Southwest slope.....	76.9	125.0	7.7	0.5	14.6	2.6
Larch fir flat <sup>a</sup> .....	75.3	115.0	7.0	2.1	13.4	5.0
White pine flat <sup>b</sup> .....	75.9	118.0	19.1	3.4	38.3	11.0

<sup>a</sup> Soil of sandy loam.

<sup>b</sup> Clay loam soil.

### SUMMARY

Large openings made in the forest cover by clear-cutting cause increase in air and soil temperature, evaporation and moisture deficit, which present unfavorable conditions for reestablishment of moisture-loving species. Furthermore, changed surface conditions resulting from large openings and vegetation on areas completely cleared may defeat natural regeneration altogether.

Surfaces of ash and bare mineral soil when loose offer the most favorable conditions for rapid germination and establishment of seedlings provided seed is immediately available, but where overhead or adjacent trees must supply the seed for natural restocking over a period of years, a loose and protected surface such as is provided by needle duff and light vegetation is more favorable. The tests show that Engelmann spruce and western larch, and in this class may be placed western red cedar and western hemlock, germinate well on duff surfaces, but have very poor survival on account of their short roots. The extremely high surface soil temperatures which occur on cleared and exposed flats and south slopes are injurious to establishment of seedlings of western white pine, cedar, and hemlock, and this explains the general scarcity of these species on sites exposed to sun and wind and the difficulty of restocking these after clear-cutting on a large scale.

A method of cutting which would provide smaller openings and partial shade or shelter would produce better silvicultural results.

<sup>2</sup> While no experimental data are available bearing on the effect of direct heat on unignified seedlings, Dr. Paul Sorauer (Manual of plant diseases, tr. by F. Dorrance. Ed. 3, v. 1, illus. [Wilkes-Barre, Pa. 1914-1922]) states that a temperature of 104 to 122° F. causes death of plant leaves by burning. Prof. J. W. Toumey and E. J. Neethling (Some effects of cover over coniferous seed beds in southern New England, Yale Univ., School Forestry Bul. 9, 39 p., illus., 1923) record having obtained surface temperatures up to 130° F. which caused death-forming lesions on the unignified seedling stems. The author has observed similar results with Engelmann spruce in the greenhouse at temperatures between 110° and 120° F.



# THE EFFECT OF CYANAMID AND RELATED COMPOUNDS ON THE NUMBER OF MICROORGANISMS IN SOIL <sup>1</sup>

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## INTRODUCTION

The decomposition of cyanamid in soil is directly dependent in certain stages of the process upon the numbers, species and efficiency of the soil microorganisms in bringing about the desired changes. The conversion of calcium cyanamide into acid cyanamide, then free cyanamide and the latter into urea is thought to be chiefly a series of chemical changes in which soil colloids play an important rôle. The conversion of urea into ammonia and the oxidation of the latter to nitrites and nitrates are biological phenomena. It is, therefore, important to know the effect of cyanamid and its possible transformation products upon the numbers of soil organisms. It was with this idea in view that the experiments reported below were undertaken.

## EXPERIMENTAL

Five groups of experiments were carried out at intervals lasting over a period of about 18 months. The methods used in the first of these were considerably different from the later work and are, therefore, discussed separately.

### EFFECT OF CYANAMID AND OTHER NITROGENOUS COMPOUNDS ON BACTERIAL NUMBERS

A Susquehanna loam soil of relatively high fertility was secured from a garden, sieved while moist, and spread out in a thin layer to dry where not exposed to direct sunlight. Sixteen samples of 500 gm. each were then weighed out into 800 cc. beakers. To each was added 5 gm. of precipitated calcium carbonate and the different nitrogenous materials shown in Table I, these materials being uniformly mixed with the soil. The optimum moisture content of the soil was then determined and this quantity, 21.5 cc. of water per 100 gm. of soil, was added regardless of treatment. It was realized that the actual quantity of available moisture would be less in the soil receiving cyanamid, because of its drying properties, but it was decided not to make a correction for this factor. During the incubation period, which began on February 5, 1921, the beakers were kept covered with watch glasses and maintained at room temperature. Water was added at frequent intervals to replace that lost by evaporation.

The first samples for analysis were taken from the center of the beakers at the end of 16 days by means of a sterile cork borer, having a diameter of about three-fourths of an inch. These soil samples were added to flasks containing sterile tap water, then shaken at intervals covering a period of a half hour and agar plates poured according to the usual bacteriological technique.

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<sup>1</sup> Received for publication April 28, 1924—issued Nov., 1924.

The medium used was Lipman and Brown's modified synthetic agar, having the following composition:

	Grams
Dextrose.....	10.0
MgSO <sub>4</sub> .....	.2
K <sub>2</sub> HPO <sub>4</sub> .....	.5
Peptone.....	.05
Agar.....	15.0
FeSO <sub>4</sub> .....	Trace.
Distilled H <sub>2</sub> O.....	1,000 cc.

The quantity of di-potassium phosphate used was just sufficient to make the reaction optimum for bacterial growth and hence no adjustment was necessary.

After an incubation period of seven days at room temperature, the number of colonies appearing on each plate was determined. It happened that the dilution used was somewhat low for the most accurate results, and furthermore, several plates were overrun by quick-growing fungi. The large number of plates poured for each treatment, namely 10 (duplicate beakers of soil and 5 plates from each beaker) largely overcame these sources of error, making the final results as accurate as may ordinarily be expected from quantitative bacteriological work. The average results for the various treatments, expressed in terms of millions of microorganisms per gram of moist soil, are given in Table I.

The second set of samples for biological analysis was taken from the beakers of soil 31 days after the beginning of the incubation period. Plates were poured as previously except that a higher dilution was used. Three plates for each dilution were poured, making a total of 12 for each soil treatment. The average figures are given in Table I, together with those from the first sampling.

From the data as given, it will be observed that there was no constant relationship between bacterial numbers as shown by the methods used and the known effects of the various materials on plant growth. All of the materials used increased the numbers of soil organisms. Of these materials, the two which are known to be the best sources of nitrogen for plants under widely varying conditions, namely, ammonium sulphate and urea, produced only moderate stimulations. In this connection, it is necessary to bear in mind that the urea bacteria do not grow well, if at all, on media such as the one used which did not contain urea. These urea bacteria probably were increased, but this could not be discovered by the method used. Dicyanodiamid, which is unavailable as a fertilizer and injurious to nitrification, produced a moderate increase in numbers. Guanylurea sulphate, another material of doubtful agricultural value, caused greater increases than either of the three materials just mentioned. Cyanamid proved to be remarkably effective in increasing the rate of multiplication of the soil organisms, this increase amounting to 57.8 per cent within 16 days and 320.2 per cent after 31 days. On the other hand, very large quantities (2 per cent) practically sterilized the soil or at least so weakened the organisms that they were unable to develop on artificial media.

TABLE I.—Numbers of microorganisms in soil following applications of cyanamid and related compounds

Treatment	Millions per gram	
	16 days	31 days
No nitrogen.....	22.3	22.8
Ammonium sulphate 0.1 per cent.....	22.5	31.7
Urea (N=0.1 per cent ammonium sulphate).....	30.9	27.2
Dicyanodiamid (N=0.1 per cent ammonium sulphate).....	29.2	25.6
Guanylurea sulphate (N=0.1 per cent ammonium sulphate).....	28.6	51.6
Cyanamid (N=0.1 per cent ammonium sulphate).....	35.2	95.8
Cyanamid (N=0.5 per cent ammonium sulphate).....	42.7	67.2
Cyanamid (N=2 per cent ammonium sulphate).....	.05	.12

FURTHER STUDIES ON THE EFFECT OF CYANAMID AND OTHER NITROGENOUS COMPOUNDS ON BACTERIAL NUMBERS

In continuation of the work discussed above, a much more comprehensive set of experiments was outlined somewhat similar to the first but using several changes in technique suggested by the first experiments. The chief of these was the difference in method of incubation of the soil samples. Instead of using a large sample of soil and taking samples from the same at intervals, a large number of small samples of 50 gm. each were incubated in 250 cc. Erlenmeyer flasks. This eliminated the many objectionable features and sources of error connected with the securing of samples. The mere addition of sterile water to the 50 gm. of soil in the flasks, followed by thorough shaking, made the first dilution.

The soil used was a Clarksville loam which had been in cultivation for several years and was rather low in fertility. To several 50 gm. portions of the air-dried soil in the flasks were added from 5 to 50 mgm. of N in the forms of ammonium sulphate, cyanamid (untreated), cyanamid (oiled and hydrated), dicyanodiamid, guanylurea sulphate, and urea. After thorough mixing, water was added at the rate of 19 cc. per 100 gm. of soil to bring the moisture content up to the optimum. In order that the soils might have better air circulation, the flasks were not plugged with cotton but kept in an incubator containing a large evaporating surface of water which kept the humidity at near saturation. A slow loss of moisture did occur, however, and this was restored at intervals. The experiment, which started on June 18, 1921, was continued at room temperature for various lengths of time, as shown in Table II.

TABLE II.—Numbers of microorganisms in soil following applications of cyanamid and related compounds

Treatment (per 50 gm. of soil)	Mgm. N	Millions per gram				
		3 days	7 days	21 days	31 days	38 days
Control		2.04	2.09	1.54	1.26	2.60
Ammonium sulphate	5	1.86	1.54	Lost.	.76	1.99
Do.	10	1.90	2.08	.82	1.25	1.77
Do.	25	2.18	2.45	.59	1.70	2.95
Do.	50	3.91	5.26	1.26	2.52	3.20
Cyanamid, untreated	5	2.58	4.08	.98	1.03	2.72
Do.	10	5.52	4.43	1.06	2.23	2.28
Do.	25	12.03	8.63	.95	1.81	3.95
Do.	50	14.57	6.90	1.22	2.81	2.19
Cyanamid, oiled and hydrated	5	2.14	4.30	1.13	1.24	2.47
Do.	10	4.68	2.65	1.41	1.58	3.37
Do.	25	13.44	7.95	1.16	1.69	3.72
Do.	50	14.53	Lost.	1.30	2.30	3.40
Dicyanodiamid	5	3.23	2.54	2.51	1.55	2.66
Do.	10	2.10	2.65	2.22	1.36	1.72
Do.	25	2.05	1.21	1.75	1.51	1.86
Do.	50	1.65	1.45	1.28	1.32	1.81
Guanylurea sulphate	5	2.00	1.28	1.28	1.40	1.96
Do.	10	2.10	1.95	1.03	1.08	1.73
Do.	25	2.15	1.43	.89	1.06	2.18
Do.	50	2.68	1.50	.93	1.64	2.63
Urea	5	1.97	1.28	.53	1.30	1.58
Do.	10	3.05	1.02	.50	1.08	1.70
Do.	25	2.76	1.25	.62	1.21	1.72
Do.	50	1.99	1.33	.52	Lost.	1.74

The pouring of the plates was done in the same manner as previously, using Lipman and Brown's synthetic agar. The average number of organisms, in terms of millions per gram of soil, is given in Table II for the different treatments. All plates which were badly overrun by fungi or were for other reasons decidedly abnormal were eliminated in determining the averages.

From the data it will be observed that ammonium sulphate produced only a slight increase in the numbers of bacteria and then only with the two largest rates of application. Likewise, dicyanodiamid, guanylurea sulphate, and urea produced no appreciable effects, the slight changes in numbers usually being within the experimental error. On the other hand, cyanamid, whether oiled and hydrated or untreated, produced an increase of approximately 700 per cent at the largest rate of application and smaller increases with lesser amounts. The maximum effect on the numbers of organisms was observed at the first analytical period, namely, 3 days. After 7 days the numbers had decreased to about half and continued to decrease until at the end of 3 weeks there were actually fewer in the treated than in the untreated soils. At the end of the experiment, 38 days, all soils regardless of the kind of fertilizer applied and even the controls showed more microorganisms than at the previous incubation period of 31 days. It is of considerable practical importance to know that the coating of oil which treated cyanamid receives does not appreciably affect the numbers of bacteria in the soil. It should be remembered, however, that most of the organisms which grow on the synthetic agar used are ammonifiers and hence the results are in no way an indication of the effect of the different materials on either the numbers of nitrifiers or the rate of the oxidation produced by them. The conditions required for the optimum growth of the two classes of organisms, especially on laboratory media, are quite different, the nitrifiers being especially susceptible to slight variations in reaction, concentration, source of food supply, and presence of undesirable substances. The ammonifiers are quite hardy and grow under widely different environments.

In comparing the results given in Tables I and II it is necessary to bear in mind the radical differences between the two sets of experiments. The first experiment was with a very fertile soil, the second with a very poor soil deficient in organic matter, nitrogen, and phosphorus. Again, the first work was conducted during the winter months with a soil taken from the field from underneath a crust of frozen soil; the second experiment was made during the hot summer months with soil secured from the field at the time. Furthermore, there were considerable differences, as already noted, in the methods of conducting the experiments, time of incubation, rates of application, etc. All work was done at room temperature which varied during the winter from 15° to 24° C., and during the summer from 21° to 37° C. We could, therefore, scarcely expect closely agreeing results between the two sets of experiments.

The rates at which the changes in the numbers occurred are very different in the two sets of experiments. For instance, in the winter work the maximum numbers were observed in the soils receiving cyanamid at the 31-day incubation period while in the summer studies the maximum numbers were found after 3 days and rapidly dwindled thereafter. There was a marked increase in numbers in both instances, except where too much of the material was used. Of the other materials, ammonium sulphate, urea, and dicyanodiamid produced slightly greater stimulations in numbers in the first set of experiments. A discrepancy occurred in the case of guanylurea sulphate, this material producing a maximum stimulation of 126 per cent with the richer soil and only about 32 per cent with the poorer soil. In fact, in the latter case, the material usually acted as entirely inert, slight depressions in numbers being about as frequent as stimulations.

A STUDY OF THE RELATION OF THE LIME CONTENT OF CYANAMID TO  
THE INCREASE IN BACTERIAL NUMBERS

Using the same technique as outlined in the preceding experiment, but using the Susquehanna loam soil, another experiment was started on March 27, 1922, in which cyanamid was applied at different rates and the numbers of bacteria developing compared with similar tests where lime was used corresponding to the amount in the cyanamid. The three common forms of lime were used, namely, the oxid, hydroxid, and carbonate, the amounts being calculated on the basis of calcium content. The numbers of colonies which developed on the plates within 4 days are reported in Table III.

TABLE III.—Numbers of microorganisms in soil following applications of cyanamid and lime

Treatment (per 50 gm. of soil)	Mgm.	Millions per gram			
		3 days	7 days	14 days	21 days
Control.....		3.64	3.01	2.17	1.61
Cyanamid.....	123	12.60	14.00	27.27	2.40
Do.....	247	8.15	17.60	24.65	7.60
Do.....	493	3.46	35.26	152.52	.40
Do.....	123	24.70	45.30	60.20	1.47
CaCO <sub>3</sub> .....	570				
Cyanamid.....	247	6.12	94.53	30.55	1.45
CaCO <sub>3</sub> .....	570				
Cyanamid.....	493	1.68	35.20	69.90	2.60
CaCO <sub>3</sub> .....	570				
Do.....	143	4.35	2.98	1.39	2.12
Do.....	285	5.82	4.08	2.07	2.13
Do.....	570	5.57	5.15	1.93	1.30
Ca(OH) <sub>2</sub> .....	105	6.05	10.42	2.63	1.17
Do.....	211	10.25	20.38	14.53	5.13
Do.....	422	9.95	82.73	30.33	3.67
CaO.....	80	7.70	8.01	2.38	1.68
Do.....	160	7.70	20.75	21.58	2.48
Do.....	319	27.05	92.80	44.40	3.57

It will be noted that cyanamid again produced a very marked increase in bacterial numbers, particularly at the largest rate of application. The time required for the maximum increase was usually 14 days, but this varied slightly with the different treatments. Even at the smallest rate of application of cyanamid, there were about 13 times as many organisms present after 14 days as in the control soil. With the largest application, the increase was 70 times, over 150 millions per gram having been determined by the plate method. Undoubtedly, several millions more were present which did not develop upon the medium used.

The use of calcium carbonate, even though it produced only a comparatively small increase in numbers when used by itself, very markedly increased the numbers when mixed with the two lower percentages of cyanamid. With the largest cyanamid application, the maximum number was 69.9 millions, as compared with 152.5 millions with cyanamid alone. It is difficult to explain this variation, but it was quite likely due to the high alkalinity.

The three forms of lime used produced results about as might have been expected. Calcium carbonate, being neutral and more or less inert, produced only a comparatively small stimulation; the hydroxid, which is basic and quick to act, increased the numbers up to 27 times that of the control within seven days; the oxid, which is still more active, gave a maximum increase of 31 times. If large applications of the oxid are used, as has been done in other experiments



not reported here, the soil is practically sterilized exactly as is the case with heavy applications of cyanamid.

This experiment was planned to determine whether the marked stimulation to bacterial numbers following applications of cyanamid can be largely explained on the basis of its lime content. The results indicate that this is the explanation for a large part of the increase, but evidently the cyanamide and its transformation products as well as the impurities in commercial cyanamid also enter into the results in a very marked manner.

THE RELATIVE IMPORTANCE OF THE CYANAMIDE AND CALCIUM OXID IN CYANAMID IN INCREASING BACTERIAL NUMBERS

In continuation of the work reported in Table III using the same methods and soil, an additional series of experiments was started on June 23, 1922, using pure cyanamide ( $H_2CN_2$ ) and calcium oxid singly and together in comparison with like applications of cyanamid basing the rates on the nitrogen and calcium contents of the cyanamid. These results are given in Table IV.

Again it will be observed that cyanamid produced a big increase in bacterial numbers. With the three lowest applications, the larger the application the greater the stimulation, but with the heaviest application the numbers were less than with the medium rates, but still far above the control. Using calcium oxid in amounts comparable to that in the cyanamid, large increases in bacterial numbers were also noted and in every case the larger the application the higher the figures. In many respects, the data corresponds to that noted for cyanamid except that the largest application of calcium oxid did not produce a falling off as was observed with cyanamid.

The effect of pure cyanamide was very peculiar. At the two smaller rates, it produced only moderate increases as compared with either cyanamid or calcium oxid, but with 50 mgm. N per 50 gm. soil, 36 times as many bacteria were present at the end of 13 days' incubation as in the control. With double this application, the soil was partially sterilized. It is quite remarkable that cyanamide, at one application produced such a marked effect, while at either slightly higher or lower rates showed only moderate effects.

TABLE IV.—Numbers of microorganisms in soil receiving cyanamid, pure cyanamide, and calcium oxid

Treatment (per 50 gm. of soil)	Millions per gram		
	5 days	13 days	21 days
Control.....	6.26	2.85	1.98
Cyanamid 5 mgm. N.....	8.03	4.13	2.64
Cyanamid 20 mgm. N.....	43.96	23.39	9.97
Cyanamid 50 mgm. N.....	50.80	54.16	5.46
Cyanamid 100 mgm. N.....	17.20	46.30	14.66
Calcium oxid 16 mgm.....	11.06	5.30	2.67
Calcium oxid 64 mgm.....	16.88	8.11	3.50
Calcium oxid 160 mgm.....	25.90	11.07	7.03
Calcium oxid 319 mgm.....	71.05	69.26	8.24
Pure cyanamide 5 mgm. N.....	6.86	3.75	2.16
Pure cyanamide 20 mgm. N.....	9.81	12.49	6.46
Pure cyanamide 50 mgm. N.....	9.21	102.84	33.10
Pure cyanamide 100 mgm. N.....	.71	.24	.46
Pure cyanamide 5 mgm. N+calcium oxid 16 mgm.....	8.43	6.93	2.87
Pure cyanamide 20 mgm. N+calcium oxid 64 mgm.....	19.87	25.43	16.16
Pure cyanamide 50 mgm. N+calcium oxid 160 mgm.....	107.08	52.66	6.76
Pure cyanamide 100 mgm. N+calcium oxid 319 mgm.....	1.47	5.70	6.14

Where cyanamide and calcium oxid were used together in the approximate proportions found in cyanamid, the figures show clearly the importance of cyanamide in determining the results. At the two smaller rates, there was a fairly marked increase in numbers, probably due more to the calcium oxid than to the cyanamide. With the third highest rate, there was the usual marked stimulation observed with both the lime and cyanamide, used singly, as well as with cyanamid. However, the maximum effects were observed at the 5-day incubation period instead of 13 days as with cyanamide. Evidently, the lime was largely responsible for hastening the bacterial multiplication. With the highest application of the combination of cyanamide and calcium oxide, there was marked retardation at first followed by a small increase over the control later. In this case, the cyanamide was the important factor and neutralized the good effect that the same application of lime produced in the absence of the cyanamide.

EFFECT OF UREA, GUANYLUREA SULPHATE, GUANIDIN NITRATE, AND BIGUANID NITRATE UPON THE NUMBERS OF BACTERIA IN SOIL

After observing the very marked effects of cyanamid on bacterial numbers, it was of interest to extend the previous studies to include some additional transformation products of cyanamide. In this connection, guanidin nitrate and biguanid nitrate were used, as well as guanylurea sulphate and urea, previously studied. All applications were based upon the nitrogen content, the rates being 25-50, and 100 mgm. N per 50 gm. soil. In addition, sodium nitrate was used singly in amounts equivalent to the nitrate contents of guanidin and biguanid nitrates at the highest rates of application. In like manner, a test was included using magnesium sulphate equivalent to the sulphate content of the largest application of guanylurea sulphate. The data secured in this experiment, started on April 20, 1922, using the Susquehanna loam soil, are given in Table V.

In marked contrast to the effect produced by cyanamide, all of the materials used in this experiment produced only slight effects. The increases and decreases noted were usually only comparatively slight and almost within the experimental error. In this particular experiment, a large proportion of these variations may be attributed to the fact that the dilution used in plating was much too small, and consequently a variation of three or four colonies on a plate made a very great difference in the final figures.

TABLE V.—Numbers of microorganisms in soils receiving nitrogen in the forms of urea, guanylurea, guanidin, and biguanid

Treatment (per 50 gm. of soil)	Mgm. N	Millions per gram	
		8 days	18 days
Control.....		1. 70	1. 20
Urea.....	25	1. 80	1. 05
Urea.....	50	1. 77	1. 98
Urea.....	100	2. 03	1. 15
Guanylurea sulphate.....	25	1. 50	1. 98
Guanylurea sulphate.....	50	1. 33	. 65
Guanylurea sulphate.....	100	1. 23	. 67
Guanidin nitrate.....	25	1. 93	1. 15
Guanidin nitrate.....	50	3. 67	. 43
Guanidin nitrate.....	100	1. 06	1. 67
Biguanid nitrate.....	25	2. 20	1. 32
Biguanid nitrate.....	50	1. 23	1. 20
Biguanid nitrate.....	100	3. 27	1. 25
Sodium nitrate $\rightleftharpoons$ NO <sub>3</sub> in biguanid nitrate.....		2. 70	1. 20
Sodium nitrate $\rightleftharpoons$ NO <sub>3</sub> in guanidin nitrate.....		2. 23	. 83
Magnesium sulphate $\rightleftharpoons$ SO <sub>4</sub> in guanylurea sulphate.....		2. 30	1. 23

## SUMMARY

This paper presents the results of laboratory studies on the effect of cyanamide and related compounds on bacterial numbers. In considering these results, it should be borne in mind that the bacterial counts were made on Lipman and Brown's synthetic agar, which, like all media, favors the growth of certain types of organisms; in this instance chiefly the ammonifiers. The use of other substrates would probably have given different results.

Cyanamid produced unusually large increases in the number of bacteria in soils, the maximum increase usually occurring within two weeks after application, but this depended upon a number of factors, chiefly the rate of application and temperature of incubation. In one instance, the number reached was 70 times the control.

In an attempt to determine the particular constituent in cyanamide primarily responsible for this rapid bacterial multiplication, it was found that both the cyanamide content and the lime were very important. Where used alone, either calcium hydroxid or calcium oxid in amounts equivalent to that found in cyanamid produced large increases in numbers. The larger the application, the larger was the stimulation within the limits of these experiments. In the case of pure cyanamide ( $\text{H}_2\text{CN}_2$ ) the two lower rates of application produced relatively small increases and the highest partially sterilized the soil. On the other hand, the intermediate rate produced a very large increase comparable to cyanamid. It is, therefore, impossible to state whether lime or cyanamide is the greater factor in explaining the effects of cyanamid. Undoubtedly, both are important in the moderate applications, but the cyanamide or its decomposition products are largely responsible for the partial sterilization with the higher applications. The enormous increases in numbers sometimes noted with certain concentrations of cyanamid can probably be attributed in large part to the cyanamide. Further work is needed to establish these points.

Urea and ammonium sulphate produced only slight effects upon bacterial numbers, even though both materials are known to be excellent sources of plant food for higher plants. As previously pointed out, the absence of effect with urea was most likely due to the fact that the medium used did not contain urea and hence did not favor the urea bacteria. In the case of ammonium sulphate, no marked stimulation could be expected since the nitrogen is already in the ammonia stage and hence not attacked by the ammonifying organisms.

The other materials, namely, dicyanodiamid, guanylurea sulphate, guanidin nitrate, and biguanid nitrate also failed to produce marked effects even though some of them are known to be injurious or at least unavallable for higher plants.

In considering the results presented above, it should be borne in mind that the quantities of materials used were far in excess of the usual field applications. For instance, 50 mgm. of nitrogen per 50 gm. of soil would be the equivalent of slightly more than 10,000 pounds of ammonium sulphate per acre (figured as 2,000,000 pounds of soil). Obviously, no such concentration would ever occur under field conditions except possibly in isolated cases where heavy fertilizations were made in the row. Applications corresponding to field practice, where the material is uniformly mixed with the soil would probably have little effect upon bacterial numbers. Further work is needed along all of the lines treated and broad generalizations are not justified from the data here presented.

# JOURNAL OF AGRICULTURAL RESEARCH

## CONTENTS

	Page
Morphology of the Honeybee Larva - - - - - JAMES A. NELSON	1167
Effect of Winter Rations on Subsequent Pasture Gains of Steers - - - E. W. SHEETS	1215
A Study of Douglas Fir Reproduction under Various Cutting Methods - - JACOB ROESER, Jr.	1233
The Isolation and Identification of Quercetin from Apple Peels - - - CHARLES E. SANDO	1243
Gamete Production in Certain Crosses with "Rogues" in Peas - - - - WILBER BROTHEARTON, Jr.	1247
Survey of Blister Rust Infection on Pines at Kittery Point, Maine, and the Effect of Ribes Eradication in Controlling the Disease - - - - - G. B. POSEY and E. R. FORD	1253

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## MORPHOLOGY OF THE HONEYBEE LARVA<sup>1</sup>

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### INTRODUCTION

The observations here recorded seem desirable for several reasons. In the first place, it has been the policy of the Office of Beekeeping Investigations to secure, as far as possible, comprehensive and detailed information regarding all phases of the life history of the honeybee. An account of the development of the honeybee in the egg already having been completed (36)<sup>2</sup>, a study of the postembryonic development appears next in order, and this involves necessarily a thorough and intimate knowledge of the structure of the larva. Moreover, an account of the structure of the bee larva ought not to be entirely without interest to intelligent beekeepers. Finally, such an account will unquestionably prove valuable to investigators of the pathology of brood diseases of the honeybee.

No complete description of the structure of the larva of the honeybee has yet been published. This is especially surprising in view of the attention bestowed on the structure of the imago. Leuckart and Nitsche (31) have included the bee larva in the series of types illustrated by their wall charts. These illustrations, however, convey but a meagre amount of information on this subject and in some respects are incorrect. Anglas (1) gives a description of the bee larva in his account of the metamorphosis of the wasp and that of the honeybee, but here again the description of the morphology of the larva is only incidental and for the most part brief and superficial. Moreover, some organ systems, such as the nervous system, are left almost untouched.

In the following account the larva has been treated as if it constituted a single stage. The larval period, it is true, embraces a series of stages, differing from one another and representing a continuous process of development, but the principal morphological features peculiar to the larva as such remain surprisingly constant. Since a sketch of the structure of the newly-hatched larva has already been published by the writer in connection with the embryonic development, and, since it is especially important to know the structural conditions immediately preceding pupation, especial attention is here paid to the larva which has virtually attained its full growth. The following descriptions therefore apply to such a larva, unless otherwise stated. Wherever important changes in larval structures accompany growth, these are taken into consideration.

### EXTERNAL FORM

In general appearance the bee larva much resembles many other cruciform larvae. When removed from the cell the full-grown larva (Pl. 1, D) is fusiform, the posterior end being the larger. Younger larvae are more slender and more nearly cylindriciform, while the head is much larger in proportion to the trunk. In those recently hatched the body differs in its proportions from the bodies of

<sup>1</sup> Received for publication Apr. 22, 1924—issued Nov., 1924.

<sup>2</sup> Reference is made by number (*italic*) to "Literature cited," pp. 1208-1212.

older larvae in that the anterior end is the larger, the body tapering toward a pointed posterior end. In old larvae the posterior end is blunt and rounded. In larvae of all ages the body is strongly flexed toward the ventral side. In marked contrast the larvae of the Vespidae have bodies which are nearly straight.

In color the larva is ivory white. As seen in the comb it appears to be pure white by contrast with the brown comb, but when removed and placed on a white surface it takes on a yellowish or brownish tinge.

A series of sharply defined constrictions divides the body into 13 segments, 3 thoracic and 10 abdominal.<sup>3</sup> In antero-posterior extent the three thoracic segments are subequal and slightly inferior to the adjacent abdominal segments. The latter are also subequal in extent, with the exception of the 9th and 10th. The 9th is short and has a somewhat discoid form, as shown in the figure. The 10th is quite small and also discoid and bears in its center a low papilla on which is situated the anal opening.

The ventral or sternal surface is marked off from the lateral surfaces by a deep groove, the ventrolateral suture (Pl. 1, D, *VLS*), which extends from the 1st to the 8th abdominal segments, being absent on the three thoracic and the last two abdominal segments. The lateral surfaces of the abdominal segments, with the exception of the last two, are raised to form rounded lobes, the epipleural lobes (*EpL*). These lobes, while sharply marked off from the ventral surface by the ventrolateral suture, merge almost imperceptibly into the dorso-lateral surfaces of the body. The epipleural lobes are often more or less confluent, producing the appearance of a beaded ridge on each side of the body. The abrupt termination of these ridges at the posterior boundary of the third thoracic segment produces the appearance of "shoulders" noted by White (50).

Ten pairs of spiracles are present, belonging as usual to the 2d and 3d thoracic and the first eight abdominal segments. They appear externally as minute circular apertures, each of which is surrounded by a narrow chitinous ring, the peritreme. They are located at the same level on all of the segments, about half way between the dorsal mid-line and the ventrolateral suture, and lie close to the anterior edges of their respective segments in depressions confluent with the intersegmental constrictions. In the thoracic region the first pair of spiracles has the appearance of belonging to the 1st instead of to the 2d thoracic segment, this appearance being due to the somewhat peculiar outline of the contiguous margins of these two segments (see fig. 4, A). Below the spiracles of the 2d and 3d thoracic segments the oval outlines of the wing rudiments (*WngR*) may be somewhat indistinctly seen. On all of the segments bearing spiracles a shallow linear depression or suture arises in the neighborhood of each spiracle and takes an oblique course dorsad and caudad across the dorso-lateral surface of the segment toward the dorsal mid-line, where it disappears, thus separating the dorsal half of the segment into anterior (*Prs*) and posterior (*Scs*) divisions. Comparison with coleopterous larvae, such as those of *Dendroctonus* (17), makes it evident that the anterior division corresponds to the prescutum, while the posterior, which is confluent laterally with the remainder of the segment, corresponds to the scutum and scutellum together, which in the coleopterous larva are separated by a suture. These divisions, and in fact the entire contour of the body, are intimately related to the internal structure, as will be more completely explained in the description of the muscular system.

The head (Pl. 1, F, G), with regard to its general outline, may be described as bluntly conical, the base of the cone being formed by the head capsule, the apex by the labrum and the other mouth parts. The head is joined to the

<sup>3</sup> Embryological evidence indicates that there are actually 12 segments represented in the abdomen of the bee larva (36, 38).

trunk by nearly its entire width, a sharp and narrow constriction representing the neck region. Normally the head is retracted into the prothoracic segment, thus forming a deep fold between the two. The ventral third of the head, including the labium, maxillae, and that part of the head capsule to which they are attached, is sharply separated from the remainder of the head, which constitutes the head capsule (*26*), by a deep furrow which extends on each side from between the bases of the mandibles and maxillae to the posterior limits of the head. This may be conveniently designated as the *lateral furrow*.<sup>4</sup> Its posterior end, on each side, is continuous with the lumen of the posterior arms of the tentorium (*2Ten*). In face view the head capsule is seen to be divided dorsally into two equal lobes by a median cleft (Pl. 1, F). On the convex surface of each of these lobes is a small rounded elevation (*Ant*), representing the distal end of the antennal rudiments. Springing from the head capsule in the mid-line, between the antennal rudiments and directed cephalad and ventrad is the cushion-shaped common rudiment of the labrum and clypeus (*Clp+Lm*). Its distal end is free from the head capsule and its tip slightly indented in the mid-line.

In *Vespa*, according to Kirmayer (*26*), the labrum is distinguishable from the clypeus, but apparently this is not always possible, since the two areas are represented as distinct in Kirmayer's figure 30, but not in his figures 28 and 29. In the bee larva they are not always readily distinguishable on the exterior, but in many preparations of the head of advanced larvæ the rudiment of the clypeus and labrum is seen to be crossed by a delicate and distinct fold, dividing it into a distal and a proximal part, which can only be labrum and clypeus, respectively (Pl. 1, F). These two parts are also evidently indicated by the arrangements of the muscles. (Pl. 6, C.)

Beneath the labrum is the mouth opening (*Mth*), on each side of which are the conical mandibles (*Md*), curved in such a way that their pointed tips lie beneath the labrum. The maxillae (*Mx*), which are attached to the ventral part of the head capsule, are situated somewhat farther apart than the mandibles. They are also longer and of larger diameter, converging a trifle toward the mid-line, cylindrical in form, slightly tapering toward their apices, which are rounded and tipped each with a small spine. The labium (*Lb*), which springs from the ventral part of the head in the mid-line, is of relatively large size, projecting beyond the other mouth parts, bluntly conical in form, rounded at the apex, on which is a transversely elongated elevation bearing the slit-like common opening of the silk glands (*SlkDO*). On each side of the latter, and slightly ventrad to it, is a small spine, similar to those on the maxillae. The upper surface of the labium is separated from the lower surface of the labrum by a narrow cleft, forming the mouth opening (*Mth*) which is bounded laterally by the mandibles and maxillae. All of the mouth parts are soft and fleshy and covered only by thin chitin, with, of course, the exception of the small spines already mentioned.

Above the common rudiment of the clypeus and labrum, on each side of the mid-line, a shallow depression is found. These depressions represent the insertions of muscles on the inner surface of the head capsule. Above each of the antennal rudiments is a smaller but somewhat more sharply marked triangular depression; these also mark points where muscles are inserted.

<sup>4</sup> A study of the figure by Kirmayer (*26*) of the head of the wasp at different stages makes it evident that the "lateral furrow" of the wasp larva corresponds very closely with the margins of the fossa of the proboscis in the imago and this of course must be also true in the honeybee. It would appear, therefore, that the ventral part of the larval head lying between the two lateral furrows becomes later differentiated into the various structures included in the fossa of the proboscis—mentum, submentum, cardines, etc., and also that part of the occipital region ventrad to the foramen.



The description above given applies to a bee larva four or five days old, after removal from the cell. The larva is never permitted to assume this form, however, until after the cell is sealed, when it undergoes the important changes preliminary to pupation. The actual shape assumed by the bee larva of from four to five days old is shown in Plate 1, E. The specimen from which this drawing was made was fixed and hardened in the normal position in the cell, from which it was afterwards extracted. As can readily be seen, the larva is bent ventrad in the shape of the letter U, head and anus being brought close together, and fits its narrow quarters so completely and so snugly that it forms a veritable cast of the interior of the cell, the prismatic six-sided form of the latter being plainly reproduced by the larva, which not only takes nourishment and grows in this compressed state, but on being withdrawn from the cell can immediately assume the plump, rounded form shown in figure 1, the only remaining perceptible evidence of its close confinement being a slight asymmetry of the 8th abdominal segment, the lateral fold on this segment being more turgid on the side turned toward the bottom of the cell. This is evident in Plate 1, E. In all the specimens of larvæ fixed and hardened within the cell the head lies somewhat closer to the mouth of the cell than the anus, the long axis of the larva thus being spirally curved. In some larvæ the right side is outermost, in others the left.

Kellogg (24) has given a brief account, accompanied by two figures, of the head and mouth parts of the honeybee in connection with a study of the metamorphosis. It is of interest to note here that the spines on the maxillæ were found to represent the minute one-segmented maxillary palpi of the adult. This may readily be seen in stained and cleared preparations of the head of larvæ about to pupate. Kirmayer (26) has given a detailed account of the structure of the head of *Vespa* in connection with the changes taking place during the metamorphosis. Kirmayer's excellent figures of the head of the larva of *Vespa* show that while it is similar to that of the honeybee, it differs much in details, and particularly in the proportions of the various parts. It may be noted that in general the head of the larva of *Vespa* is both shorter and narrower than that of the bee larva, the labrum being indented ventrad and even a trifle caudad. (Pl. 10, A.) The labium is relatively much smaller, while the head capsule lacks the conspicuous median dorsal cleft seen in the honeybee.

### HYPODERMIS AND CUTICLE

The hypodermis, which, together with the cuticle secreted by it constitutes the body wall, is a simple one-layered epithelium, differing in thickness in different parts of the larva. These differences are indicated in many of the illustrations, but it may be said that in general both hypodermis and cuticle are thin over the entire extent of the trunk, the hypodermis being as a rule thinner at the middle of the segments and increasing in thickness in the intersegmented regions, and therefore ranging from the squamous to the cuboidal type. In the head the hypodermis has a considerably greater average thickness than in the trunk (Pl. 2, B, and 3, D). The hypodermal cells here have as a rule a prismatic form, and are in many places so slender and so closely crowded together that the nuclei are compelled to lie at different levels, giving the impression of a many-layered epithelium. It should be noted that in the larva there is no thinning of the hypodermis or cuticle at the borders of the segments or at the bases of the mouth parts. Such a thinning would correspond to the articular membranes of the imago.

The rudiments of the antennæ, wings, legs, and genitalia remain to be mentioned, since they are hypodermal structures. The antennal rudiments of old larvæ are ovoid in form and are situated in deep depressions or cavities (peripodal

cavities) on either side of the labrum. They are directed cephalad and slightly mesiad, their smaller ends directed outward, and are attached by the mesial side of their larger inner ends to the head capsule on the mesial side of the peripodal cavities. The long axis of the rudiments is therefore actually bent through an angle of about 90 degrees (Pl. 2, D). The cavities themselves are closed only by the chitinous cuticle, which is continuous over their external openings. The tips of the antennal rudiments project slightly above the general surface of the head and produce the small rounded elevations visible from the exterior (Pl. 1, F and G, *Ant*). The segments of the antennæ are indicated by the wavy contour of their hypodermis as well as by the distribution of the antennal nerve. (Pl. 2, D, *AntNv*.)

The leg rudiments (fig. 1 and 4, A, *1L-3L*) are short fusiform or ovoid in shape, like the antennæ, and are also situated in deep open depressions covered externally only by the cuticle. The leg rudiments are directed caudad and mesiad. The segments of the imaginal legs are indicated by annular constrictions. The wing rudiments are flat hollow outgrowths of the hypodermis of the mesothoracic and metathoracic segments and are situated in shallow depressions low down on these segments, close to the ventral surface and at a considerable distance ventrad to the stigmata of these segments (fig. 1, Pl. 1, D, and fig. 4, A, *WngR*). In outline the wing rudiments are heart-shaped.

The rudiments of the genitalia (fig. 1, *1G-3G*) are six in number, one pair being situated on the sternite of the 8th abdominal segment and two pairs on the 9th abdominal segment, as described by Dewitz (9). At this stage they are small knob-like outgrowths situated in open depressions.

While the cuticle of the trunk is thin and elastic, that covering the cranium is much thicker and forms a more or less rigid capsule for the protection of the contained brain and other organs. The chitin covering the mouth parts is, as has already been said, thin and relatively flexible.

The rigidity of the head capsule is further increased by the cranial endoskeleton or tentorium, formed during the embryonic period by invaginations of the ectoderm (36). In the imagoes of insects the tentorium is evident as a framework of chitinous bars, but in the bee larva the embryonic conditions persist so that the tentorium consists of tubular ingrowths of the hypodermis lined with chitin, the hypodermal portion being relatively well developed as compared with the imago, in which the hypodermis forms a relatively insignificant layer. Lying between the oesophagus and the suboesophageal ganglion, transverse to the long axis, is a wide tube lined with a thick layer of chitin, compressed in a dorsoventral direction and slightly curved, the convex side being directed cephalad. This tube is subdivided—on embryological evidence—into three nearly equal parts, a central body (Pl. 1, A, and 6, D, *Ten*), and two lateral arms, the "posterior" arms (*2Ten*). The latter become continuous with the walls of the head capsule at the posterior ends of the deep lateral furrows of the head. These infoldings, or at least their posterior part, may in fact be considered as the expanded distal ends of the posterior arms, the furrows leading at their posterior ends directly into the lumen of the posterior arms, which, together with the lumen of the central body, forms a continuous open passageway from one side of the head to the other. At the junction of the central body with the lateral arms arise the anterior arms (Pl. 1, A, *1Ten*) which extend cephalad and dorsad to the anterior wall of the head capsule, with which they are united. These points of junction are marked on the exterior by two minute pits, situated above the bases of the mandible at the sides of the labrum (Pl. 1, F, *1Ten*). The anterior arms are also tubular but are much more slender than the posterior arms and are round in section. Halfway between their anterior and posterior ends each of the anterior arms gives off a

slender spur dorsad and laterad. These serve as the tendonous insertions of short muscles inserted on the anterior wall of the cranial capsule.

The mandibular apodemes also claim attention here. These are slender tapering chitinous spines which arise from the base of each mandible on its mesial side and are directed caudad and dorsad (Pl. 1, A, *RAp*). These, as their name indicates, serve as tendonous insertions for the large adductors of the mandibles. These spines are hollow for a considerable part of their length and are both accompanied and produced by corresponding involutions of the hypodermis.

White (50) has already called attention to the minute spines of the larval cuticle. These are more or less irregularly scattered over the surface of the cuticle of the head and trunk, the distance between them varying from 5 to 30 microns. Their length does not exceed the thickness of the cuticle, which is 5 to 6 microns. On the mesial surface of the tips of the maxillae these spines are however quite numerous, while the extreme tip of the labrum is clothed with thick-set chitinous spines, longer and more slender than those found elsewhere.

The head capsule and endoskeleton of the head of the bee are, except for slight differences in proportion, identical with those of the larva of *Vespa*, as described by Kirmayer (26).

### NERVOUS SYSTEM

The general features of the nervous system of the bee larva have already been described in an earlier publication (36). These features indicate on the whole a conformity to the simple type found in many insect larvae. In the bee larva this simple type persists throughout larval existence, although histological changes are continually taking place, especially in the brain, in the direction of the imaginal condition. In comparison with certain other insect larvae, such as *Corydalis* (13), the nervous system of the bee seems deficient, particularly with regard to the sympathetic nervous system. This may, however, only argue a deficiency in observation. The small size of the bee larva and the abundance of fat tissue, which more or less completely fills all the spaces of the body cavity, clinging tenaciously to whatever organs lie adjacent, constitute no small obstacle to successful dissection, while the tracing of nerves in sections has not been found satisfactory.

The nervous system of the bee larva has never been described or illustrated in detail. Probably the only figures worth mentioning are those of Brandt (5), and the one shown in the Leuckart and Nitsche wall charts (31), a figure widely copied in text books and evidently borrowed from Brandt. These show little more than the general plan of the nervous system, the shape and proportions of the brain and circumoesophageal connections being incorrectly represented. Brandt's figure shows correctly the number and relative size of the ganglia in the ventral chain, and also the paired connections and lateral nerves.

In describing the nervous system it may be conveniently divided into the brain, the ventral nerve cord, and the stomatogastric nervous system. The following description, unless otherwise stated, is to be understood as applying to the mature, or nearly mature, larva.

### BRAIN

The brain is of relatively large size, almost completely filling the upper part of the head capsule (fig. 1, *Br*; Pl. 1, B, C). It is divided symmetrically into two expanded crescentic halves united on their convex borders by the supraoesophageal commissure (Pl. 1, B, *Sup Com*). Seen in face view, each half presents a mesial pyriform division, the slender inferior ends of which form the crura cerebri (*CCer*) connecting the brain with the suboesophageal ganglion (*SoeGng*); above these follow in order the small but prominent tritocerebrum (*3Br*), the

deutocerebrum, olfactory or antennal lobes (*AntL*), and the large protocerebral lobes (*1Br*). Laterad and caudad the protocerebral lobes merge into the broad auriculate optic lobes (*OpL*), which lie almost precisely in the transverse plane. Their edges are quite thin and their posterior faces almost perfectly flat. The two lobes of the tritocerebrum are recognizable externally as distinct swellings most evident in profile view (Pl. 1, C, 3Br); those of the deutocerebrum, however, are to be identified externally only by the antennal nerves which spring from them. On the anterior faces of each of the optic lobes, near their ventral margins, and involving also the antennal lobes, is a well-marked depression, of a somewhat hemispherical outline. These depressions are produced by the peripodal cavities of the antennal rudiments, which in mature larvae are of large size.

Two pairs of nerve trunks arise from the brain. The first of these, the antennal nerves (Pl. 1, B and C, *AntNv*) spring from the antero-lateral faces of the antennal lobes. They then take a lateral course to the base of the antennal rudiment, where each divides to form three branches. Two of these branches innervate the adjacent masses of mesodermal cells surrounding the base of the antennal rudiment, and are therefore evidently motor nerves. The third or sensory branch enters the cavity of the antenna and passes along its lateral margin, sending off

a tuft of nerve fibers to each segment. This is well illustrated by Plate 2, D, which shows the antennal rudiment in longitudinal section with the nerve (*AntNv*) giving off branches to each of the six segments seen in the section. The labro-frontal nerve (Pl. 1, B and C, and 2, B, *LmNv*) springs from the anterior face of each of the two halves of the tritocerebrum. This nerve is, as its name

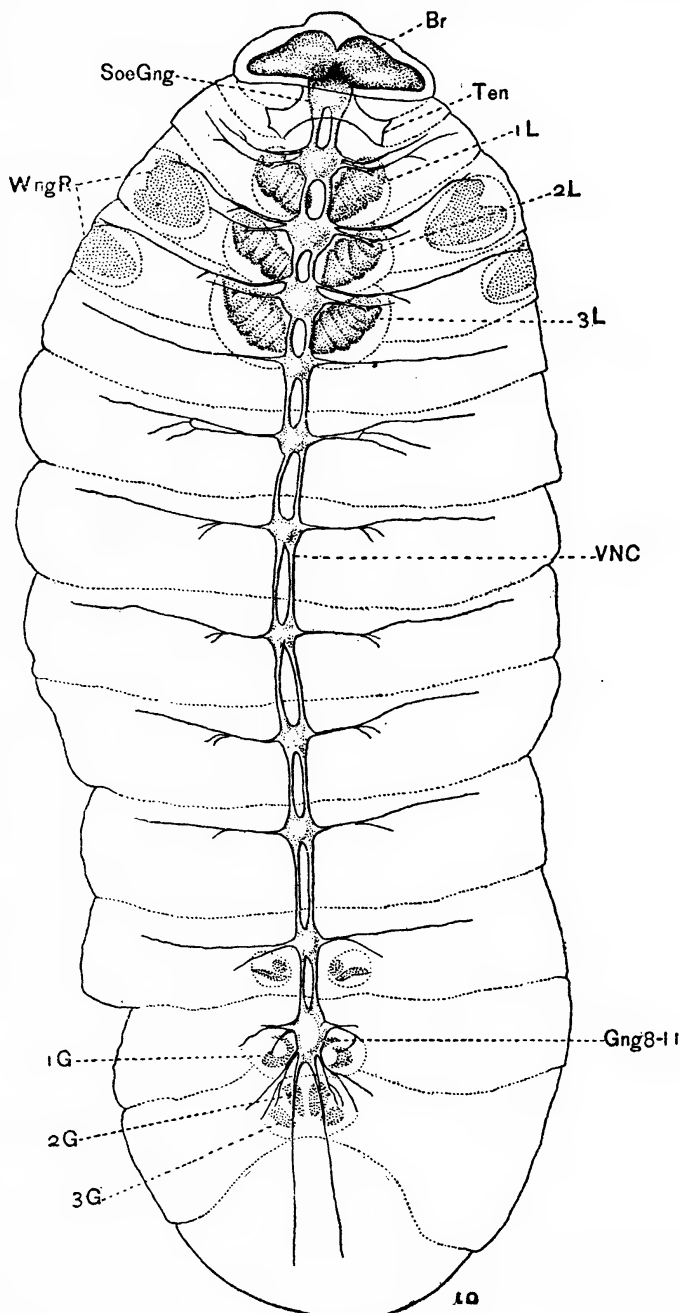


FIG. 1.—Nervous system and ventral body wall of mature honeybee larva, drawn from a dissection.  $\times 16$

indicates, a double one, and near its point of origin it divides into its component parts, the frontal nerve (Pl. 1, B and C, *FtNv*), which turns mesiad to unite with the stomatogastric or frontal ganglion (*FtGng*), and the labral nerve (*LmNv*). The latter takes a cephalad course, entering the clypeus at its extreme lateral border, and runs close under the hypodermis to the tip of the labrum, where it divides into several branches innervating the hypodermis.

The two halves of the tritocerebrum are united by the suboesophageal commissure (*SoeCom*), a relatively thick strand of nerve tissue which forms a loop below the oesophagus. Its two ends are joined to the two halves of the tritocerebrum on their ventral sides, just anterior to the crura cerebri.

In the imago of the honeybee two other pairs of nerves have been described which, however, appear to be absent in the larva. These are the tritocerebral nerve, discovered by Janet (19) in the ant *Lasius*, which issues from the inner faces of the tritocerebral lobes and innervates the inferior dilator muscles of the pharynx, and the salivary nerve (Kenyon, 25), which also issues from the tritocerebrum and innervates the salivary gland. Jonescu (21) has shown that the roots of both of these nerves lie in the suboesophageal ganglion.

The suboesophageal ganglion (Pl. 1, B and C, 2, B, and 3, A, *SoeGng*) is lenticular in form, somewhat broader at its anterior end, and turned obliquely with respect to the long axis of the larva, its anterior end being directed dorsad as well as cephalad to meet the crura cerebri. It gives rise to four pairs of nerves, as follows:

1. The mandibular nerves (Pl. 1, B and C, *MdNv*). These arise from the antero-lateral angles of the ganglion, just below the crura cerebri. Each of these nerves pursues a cephalad course, turning slightly dorsad and laterad, just below and parallel to the anterior arms of the tentorium, to the base of the mandible, where it divides into branches which are distributed to this appendage.

2. The maxillary nerves (Pl. 1, B and C, *MxNv*). These arise from the ventro-lateral face of the ganglion, a short distance behind the mandibular nerves. These nerves run ventrad, laterad, and cephalad to the anterior surface of the depressors of the maxillae (see p. 1190). Here each of these nerves divides into three branches, one of which supplies the depressor, a second the flexor of the maxilla, while the third pursues a cephalad course into the interior of the maxilla, where it branches among the mesoderm cells situated there.

3. The labial nerves (Pl. 1, B and C, *LbNv*). These arise from the ventral face of the suboesophageal ganglion, near its lateral borders, and about two-thirds of its length from the anterior end. Each of these nerves runs ventrad and laterad to the major retractor, where each nerve divides into two branches, one of which supplies the retractor muscle while the other continues cephalad to the tip of the labium..

4. X nerves of Jonescu (Pl. 1, C, *x*). These arise from the lateral faces of the ganglion close to its posterior end. Although taking their rise from prominent papillate elevations on the side of the ganglion, these nerves in old larvae are excessively slender and correspondingly difficult to trace. The evidence obtained by examination of several series of sections seems, however, to indicate that the X nerves pursue a fairly direct course ventrad and laterad, reaching the hypodermis close to the origin of the major retractors of the labium. In sections of recently hatched larvae these nerves are relatively much larger than in older larvae and in fact are very easily distinguished. Their course is directly laterad to the ventral margin of the junction of the posterior arms of the tentorium with the hypodermis of the neck region. No indication of a connection with the silk gland was seen. It appears therefore that in the bee larva this nerve is purely sensory. Jonescu (21) has described this nerve in his account of the brain of the imaginal honeybee, but is silent regarding its course and destination. The

writer has also seen it in sections of material of the imaginal bee, but has been unable to follow it to its termination. As in the larva, it is insignificant in size, although plainly evident at its point of departure from the ganglion. Janet (18) has described a fourth pair of nerves arising from the suboesophageal ganglion in the ant *Myrmica rubra*, and Krauss (28) and Hammar (13) have described a pair of nerves in the *Corydalis* larva arising from the caudo-lateral portion of the ganglion just cephalad of the junction of the connectives with the ganglion. In *Myrmica rubra* the nerve corresponding in position to the X nerve, termed by Janet "the labial sympathetic nerve (*nervus sympatheticus labi*)" gives rise to a complex system consisting of a ganglion and three branches on each side of the mid-line. These innervate the labial gland and also adjacent muscles and tracheae. In *Melanoplus* (39), *Corydalis* larva (13), *Forficula* and certain other insects (29) a pair of nerves make their exit from the lateral faces of the suboesophageal ganglion near its posterior end, generally known as "salivary gland" nerves. In the bee larva no connection between the X nerves and the salivary (silk) gland could be established, but it nevertheless is possible in view of the close correspondence in the point of exit from the suboesophageal ganglion of the salivary gland nerves in *Melanoplus*, *Corydalis*, etc., the labial sympathetic nerves in *Lasius*, and the X nerves in *Apis*, that these are all homologous. Pietschker (42) has described a nerve in the ant which he terms "*nervus accessorius*." It springs from the suboesophageal ganglion about half way between the labial nerve and the connectives and runs toward the labium, where it ends in depressions of the hypodermis, possibly representing sense organs. This nerve in its point of exit and its termination closely approximates the X nerve of the bee larva. The accessory nerve of Pietschker is entirely distinct from the salivary nerve which was also observed and makes its exit from the dorsal side of the ganglion. It is also not to be identified with the labial sympathetic nerve of Janet.

The brain and suboesophageal ganglion of the mature larva, with reference to their external form, differ slightly from those of the young larva, and very materially from those of the imago. In the newly hatched larva (36) the two lobes of the protocerebrum (including the optic lobes), as compared with those of the mature larva, are shorter and thicker, and are bent caudad instead of lying in a plane transverse to the long axis of the larva. Moreover, they show clearly a subdivision into three lobes, as described by Viallanes (49) for Orthoptera. The antennal lobes, although not prominent, are present as distinct swellings, while the tritocerebral lobes are scarcely differentiated externally from the antennal lobes on the one hand and the thick crura cerebri on the other. The latter merge caudad into the elongate suboesophageal ganglion, which is divided externally into three swellings representing its three component pairs of ganglia.

As compared with the brain of the larva that of the imago (21) shows striking differences. Superficially there is a certain resemblance between the two, principally on account of their flat expanded form and the large size of the optic lobes. In the imago, however, the protocerebral lobes become high bilobed elevations separated from the optic lobes by well marked constrictions, and the antennal lobes become conspicuous rounded swellings projecting from the anterior face of the brain; while the tritocerebral lobes, which are fairly distinct in the larva, become almost indistinguishable. Moreover, in the imago the suboesophageal commissure becomes so fused with the suboesophageal ganglion as to be indistinguishable externally, while the frontal ganglion sinks to insignificant proportions. On the other hand, the suboesophageal ganglion of the mature larva differs but little in form, size and general external appearance from that of the imago. It is scarcely necessary to remark that most of these differences, such as the difference in size of the antennal lobes, may readily be

correlated with the differences in the environmental conditions of the two forms, yet some of these differences, such as the reduction in size and relative importance of the tritocerebrum and of the suboesophageal commissure, represent developments in the direction of specialization. In other words, notwithstanding its relatively specialized form, the brain of the larva is appreciably nearer the primitive type, as represented in the lower orders of insects, than is that of the imago.

#### VENTRAL NERVE CORD

The ventral cord consists of 11 ganglia united by rather widely separated connectives (fig. 1, VNC). All of these ganglia, with the exception of the eleventh, or terminal ganglion, are situated in the anterior half of their respective segments. In form the ganglia are more or less lenticular, the first three, or thoracic, being much larger than the abdominal ganglia. The latter are subequal in size, with the exception of the eleventh or terminal ganglion, which is elongate in form and much larger than the others. Unlike the other ganglia, this one is situated in the middle of its segment, the eighth abdominal. This ganglion, as usual, is compound, consisting of three ganglia and the rudiment of a fourth (6, 38), representing, respectively, the neuromeres of the 8th, 9th, 10th and 11th abdominal segments.

Each thoracic ganglion gives off two pairs of lateral nerves (fig. 1 and 4, A). The first pair arises from the antero-lateral margin of the ganglion close to the point where the latter joins the connectives. In the prothorax these nerves run laterad in the anterior half of the segment, close to the hypodermis. In the mesothorax and metathorax these nerves also run laterad, giving off a branch to the ventral trunk muscles, and, continuing onward, skirt the anterior margin of the wing rudiments. At the antero-lateral border of the latter each of these again bifurcates, one branch going to the wing rudiment while the other passes dorsad and laterad to the viscera.

The second of the two pairs of nerves given off arises from the lateral margin of the ganglion about midway of its length. The course and distribution of these nerves is the same in all three thoracic segments. Each passes laterad and slightly caudad to the leg rudiment (1L-3L) of the corresponding segment, sending a branch to the base of the rudiment. It then continues laterad and is lost to view among the muscles and fat cells.

Each of the abdominal ganglia, exclusive of the terminal ganglion, possesses but one pair of lateral nerves (fig. 1 and 4, B, LNv). These nerves run directly laterad, giving off branches to the ventral trunk muscles, as shown in Figure 4, B, and become lost to sight among the trunk muscles and viscera.

The 8th abdominal or terminal ganglion gives off four pairs of nerves (fig. 1). The first pair arises from the lateral margins of the ganglion near its anterior end and passes laterad to the muscles and viscera of the 8th abdominal segment. The second pair of lateral nerves originates so close behind the first pair that these two pairs can be regarded as having a common root. These two pairs of nerves go to the first pair of genital rudiments (1G). The third pair of lateral nerves runs caudad into the 9th abdominal segment, breaking up meanwhile into several branches, some of which supply the second pair of genital rudiments (2G). The fourth and last pair arises from the posterior margin of the ganglion, and these nerves run almost directly caudad to the 10th segment, where they break up into branches supplying the viscera and muscles of this segment. Three segments, the 8th, 9th, and 10th abdominal, are therefore represented in the terminal ganglion on the basis of the number and distribution of the lateral nerves. The fourth segment, the 11th abdominal, is represented by only an insignificant ganglionic rudiment, and its corresponding segment disappears about the time embryonic development is completed.

## STOMATOGASTRIC NERVOUS SYSTEM

In the bee larva, so far as known, this is the sole representative of the so-called sympathetic nervous system. It consists of the frontal or stomatogastric ganglion and the nerves associated with it. The frontal ganglion is of relatively large size, in comparison with the imago, and is more or less pyriform in shape, its smaller end directed caudad (Pl. 1, B and C, and 3, A, *FtGng*). It is situated above the oesophagus and just in front of the brain. Its sole connection with the brain is made by means of the frontal commissures or nerves (*FtNv*), which are rather stout bundles of fibers arising from the two sides of the ganglion near its anterior end. From this point they run laterad and caudad to the anterior faces of the two halves of the tritocerebrum, joining there the roots of the labral nerves, as already described. From the anterior end of the frontal ganglion a small nerve is sent off, sometimes called the "frontal nerve." This term, however, is objectionable, since it is also commonly applied to the commissures connecting the frontal ganglion with the tritocerebrum. The nerve in question (Pl. 3, A, *SPhyNv*) runs cephalad along the dorsal surface of the oesophagus to the epipharynx, where it divides into several branches, some of which go to the epipharyngeal muscles. Twigs are also apparently sent to the tip of the labrum. In view of its terminations, Janet (18) has termed the corresponding nerve in *Myrmica rubra* "nerf des muscles supérieurs du pharynx situés en avant du ganglion frontal." This descriptive term the writer for convenience ventures to abbreviate to "superior pharyngeal nerve" (*SPhyNv*). In *Myrmica rubra* there is another nerve to the posterior superior muscles of the pharynx. This nerve apparently is not represented in the bee larva. The posterior smaller end of the frontal ganglion is continued caudad as the stomatogastric or recurrent nerve (Pl. 3, A, *StgNv*). This nerve passes caudad in close contact with the dorsal wall of the oesophagus, meanwhile rapidly diminishing in caliber, to the posterior part of the head. Here it bifurcates, and a little farther caudad the two forks break up into branches too small to be successfully traced. In young larvae, and even in those as much as three days old, the two bifurcations of the recurrent nerve lead to flat discoid cell masses, closely applied to the sides of the oesophagus. These cell masses have been interpreted as the pharyngeal ganglia (36). In mature larvae they are no longer present, their places being taken by branches of the recurrent nerve, as stated above.

## HISTOLOGY

The histology of the nervous system of the bee is too complex to be treated here in detail, but there are nevertheless certain outstanding features which deserve consideration.

The outer neurilemma or sheath consists of a layer of cells, usually single, limited on each side by a delicate but distinct membrane, and forms the outer covering layer of the brain, the various ganglia, the connectives, and at least the roots of the peripheral nerves. In both its thickness and in the form of its component cells the neurilemma differs much in character at different points. On the dorsal surface of the suboesophageal ganglion, for example (Pl. 2, B, *SoeGng*) the neurilemma (*Nlm*) often has the appearance of being only a single membrane, the two component membranes being actually brought very close together and the neurilemma cells being thin, flat, and spaced widely apart. At certain other points, in particular the posterior surface of the protocerebral lobes (Pl. 2, B, *Nlm*), the neurilemma is, on the other hand, very thick, and its cells, irregular and mesenchymatous in form, are seemingly disposed in several layers. Usually the neurilemma cells form a regular epithelial layer (Pl. 3, B and C, *Nlm*). Their cytoplasm, however, commonly presents a more or less shrunken and vacuolated



appearance, while the nuclei frequently stain very intensely with iron haematoxylin (Pl. 3, C, *Nlm*). An inner neurilemma is present as a more or less regular layer of small cells lying between the ganglion cells and the fibrous substance. It is best developed and most early seen in the brain (Pl. 2, B, *INlm*), and less so in the smaller ganglia. (Pl. 3, C, *INlm*.)

The brain and other parts of the central nervous system present in sections the usual core of nerve fibers and the cortical zone of nerve cells. (Pl. 2, A and B.) In the larval brain many of the features of the imaginal brain are plainly recognizable. This is particularly true of the broadly expanded optic lobes, which in transverse sections clearly display a division into inner, middle, and outer fibrillar masses (Pl. 2, A, *fm3*, *fm2*, *fm1*), while the cells and their fibrous processes show a distinct radial arrangement. In Plate 2, A, a layer of fibers intermingled with small cells is seen situated near the lateral faces of the optic lobes, between the latter and the hypodermis. These are evidently the developing postretinal fibers (*PrF*). They enter the optic lobes on the posterior surface of the latter, near their lateral margins, and may be traced around their lateral margins to the optic plates. The latter comprise two crescentic thickened areas of the hypodermis (ectoderm), one on each side of the head, their extent and relation to the remainder of the head capsule being indicated by Plate 2, C, *OpPl*.

The ocelli are present at this stage as hypodermal thickenings. Those for the two lateral ocelli are shown in Plate 2, A, *O*. The rudiment of the median ocellus lies farther cephalad, in the median groove (*43*).

Centrad and somewhat cephalad of the optic lobes, the rounded protocerebral lobes (Pl. 1, B, *1Br*) are seen to contain well-developed mushroom bodies, which are essentially similar to those of the imago (Pl. 2, B, *MB*). These bodies were found to be recognizable in a larva three days old.<sup>5</sup>

The deutocerebral or antennal lobes, although not evident externally, are distinguishable in sections by corresponding enlargements of the fibrous core (Pl. 2, A and B, *AntL*), and the relatively prominent tritocerebral lobes are similarly characterized (*3Br*).

The internal structure of the ganglia of the ventral nerve cord of the bee larva is simple, offering no striking peculiarities, and may probably be considered as fairly typical of the condition generally found in the simpler types of insect larvae. As seen in Plate 3, B, which represents a transverse section through the fifth abdominal ganglion, each ganglion is divided externally into right and left halves, representing the two members of the pair of primitive single ganglia. The outer surface of the ganglion is covered by a well-defined neurilemma (*Nlm*). Internally each lateral half consists of a central mass of nerve fibers, continuous with that of the connectives and surrounded by ganglion cells (*NvF*). These two central masses of nerve fibers are connected with one another by two parallel transverse strands of nerve fibers, the commissures (Pl. 3, B and C, *Com*). These are, as shown in Plate 3, C, situated somewhat cephalad of the middle of the ganglion. On the left side, in Plate 3, B, a strand of fibers (*LNvF*) is seen passing laterad from the central mass of fibers. On the right side the ganglion is seen to extend itself laterally into the lateral nerve of this side, in which is seen a strand of fibers (*LNvF*) corresponding to that of the opposite side. On the right side, however, the connection of the nerve fibers of the lateral nerve with the central mass is not shown in this section.

<sup>5</sup> The duration of the feeding period of the larva is from four and one-half to five and one-half days. Larvae taken at or near the end of this period are considered mature, and it is to such larvae that the description above applies, unless otherwise stated.

The suboesophageal and the terminal, or 8th, abdominal ganglia are, as already mentioned, compound ganglia. The threefold nature of the suboesophageal ganglion is evident in sagittal sections such as that represented in Plate 3, A. At first glance four segments appear to be indicated by the presence of four commissures and four corresponding groups of ganglion cells. Examination of other sections of this series, however, as well as of the sagittal sections of other larvae of the same age, and also of younger larvae, shows that the last two bundles of transverse nerve fibers, together with the last two cell groups, belong to the labial segment. This component of the suboesophageal ganglion shows from the first two well-defined transverse commissures, like those of the succeeding trunk ganglia (36, fig. 45), while the mandibular and maxillary divisions never show clearly more than one. In the terminal ganglion three double commissures are distinguishable, being those of the 8th, 9th, and 10th abdominal segments (38). The same condition is found in embryos of *Forficula* (14, fig. 44).

One detail in the cellular composition of the nervous system should be noted. In the peripheral region of the brain and ganglia of the ventral cord of mature larvae are to be seen here and there cells of large size, which in their mode of division and other characters are plainly to be identified as neuroblasts. This observation corresponds with those of Bauer (2) on the larvae of *Vespa*, *Formica*, and other insects. Hilton (15) also reports the presence of neuroblasts in the brain and ventral cord of the larva of *Corydalus*.

#### CORPORA ALLATA

The corpora or so-called "ganglia" allata have, as is now well known, no intimate connection or relation with the nervous system, but since they have also no connection with other organ systems it is convenient to follow the time-honored custom of considering them at this point. They have in the mature larva the same structures and relations as in the newly hatched larva (36). They are spherical bodies (Pl. 2, B, *CorAll*), about 0.85 mm. in diameter, situated one on each side of the mid-line, close behind the brain, lying on the dorsal side of the anterior arms of the tentorium, near their posterior ends, and in close contact with the lateral walls of the aorta. Each is composed of a compact mass of polyhedral cells having finely granular and deep-staining cytoplasm, and nuclei with abundant chromatin in the form of subspherical granules. Surrounding the corpora is a delicate membrane secreted by the outer layer of cells. Just cephalad of the corpora, on each side of the aorta, is a tuft of tracheoles. Two or three of these enter the corpora allata and may be seen here and there between the cells of the latter.

#### TRACHEAL SYSTEM

The external openings of the tracheal system, the spiracles (fig. 2, A, Pl. 1, D, *Sp*), are 20 in number, or 10 pairs, each of the 10 segments from the 2d trunk (thoracic) segment to the 10th (8th abdominal), inclusive, bearing one pair. They are arranged on each side in a row parallel with the long axis of the body, and very nearly midway between the ventral and dorsal mid-lines. The mesothoracic spiracles are placed near the anterior limits of that segment; in the older larvæ they are located so far cephalad that they have frequently the appearance of coming within the limits of the prothorax. The spiracles of the succeeding segments are also located near the anterior borders of their respective segments at the bottom of shallow depressions, which are continuous with the adjacent intersegmental constrictions. Each spiracle is a minute, round aperture situated in the middle of a small circular elevation of the surface, the stigma. The spiracles are connected by short branches, the spiracular branches (Pl. 3, D,

*SpBr*), to the tracheal trunk (fig. 2, A, *TraTr*) of the corresponding side. Each of these trunks traverses the body cavity, about midway between the hypodermis and mid-intestine, slightly ventrad to the line formed by the spiracles of that side. The tracheal trunk of a mature larva has an inside diameter of about 0.1 mm. in its posterior and larger half; in the three anterior segments its diameter is reduced to about one-half of this caliber. The anterior ends of the tracheal trunks of opposite sides meet to form a loop, the anterior transverse commissure (fig. 2, A and B, *ATraL*), in the region of the neck and above the oesophagus; a similar loop, the posterior transverse commissure (fig. 2, A, *PTraL*), is formed by the posterior ends of the tracheal trunks. This latter, however, is situated ventrad of the posterior end of the mid-intestine, near its junction with the hind intestine. The two tracheal trunks are also united by a segmentally arranged series of transverse commissures, which lie in the ventral half of the body cavity, close to the body wall, passing below the ventral nerve cord. In a larva three days old or more the prothorax is seen (fig. 2, A) to possess one commissure, while the mesothoracic, metathoracic, and first abdominal (propodeal) segments each possess two commissures. These, however, become confluent just before their junction with the tracheal trunks. It should be noted that the commissures in the thoracic segments are quite slender, while those in the 1st abdominal segment approximate the diameter of the tracheal trunks at this point. In the 2d to the 8th abdominal segments the transverse commissures are single and of large diameter, the most anterior being largest, while the transverse commissure of the 9th abdominal segment is relatively slender and its course somewhat oblique. The prothoracic commissure joins the tracheal trunks just in front of the branches from the mesothoracic spiracles, those of the mesothorax just behind these branches. The commissures of the other segments, except that of the 9th segment, join the tracheal trunks either close to or slightly caudad to the corresponding spiracular branches.

Throughout their length the tracheal trunks give off numerous branches which supply the various regions of the body. Three branches on each side supply the head. The more anterior of these (fig. 2, B, *Bra1*) arises from the anterior transverse commissure close to the mid-line and runs cephalad to the brain, passing beneath the base of the optic lobes. It then turns abruptly dorsad, and supplies the anterior face of the brain and the region immediately adjacent (*a*). In the latter part of its course it passes close to and in contact with the spine, which arises from the anterior arm of the tentorium on each side. The second branch (*Bra2*) is much larger and arises from the anterior face of the transverse commissure a short distance ectad of the branch just described. Near its base it gives off a secondary branch (*b*) which passes mesiad and cephalad above the brain between the two halves of the protocerebrum, and supplies the aorta and the brain in the region of the mushroom bodies. The main branch takes a straight course cephalad and slightly ventrad, below the optic lobe, to the anterior region of the head, where it divides into three secondary branches. One of these (*c*) curves abruptly dorsad and breaks up into a large number of tracheoles at the base of the antennal rudiment. Before terminating its course, however, it gives off a twig to the mandible and one to the labrum. The second secondary branch passes mesiad to the head cavity underlying the supraoesophageal ganglion. The third secondary branch (*d*) supplies the maxilla.

In the prothorax the only tracheal branch worth noting is one (fig. 2, B, *e*) which arises on each side from the single tracheal commissure of this segment near its junction with the tracheal trunk, and passes mesiad and cephalad to supply the walls of the salivary glands in the region of the neck. In the remaining trunk segments, exclusive of the 9th and 10th abdominal, the number and arrangement of the tracheal branches is virtually identical. Springing from the

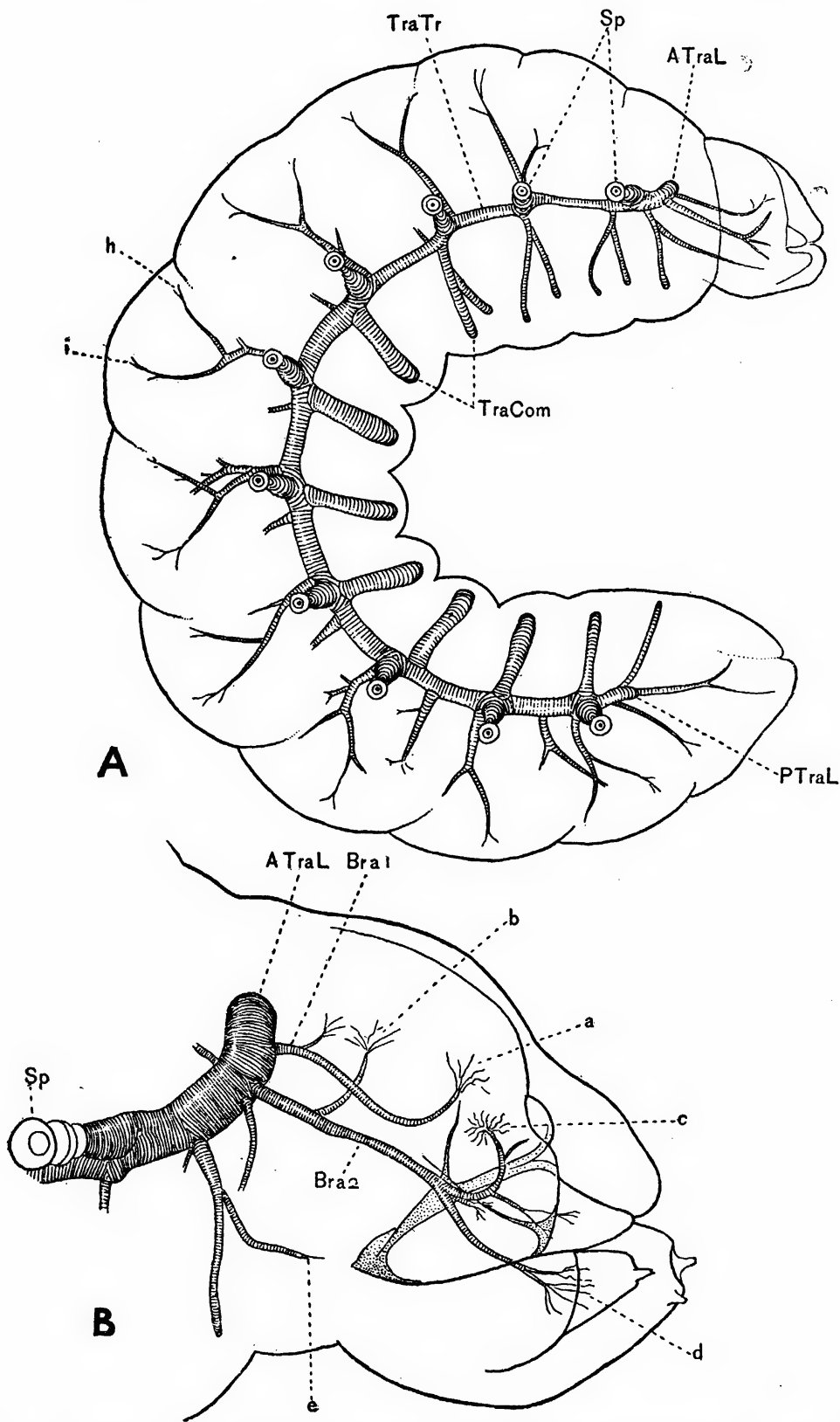


FIG. 2.—A, honeybee larva treated with caustic potash, showing tracheal system, side view,  $\times 18$ ; B, head of larva treated with caustic potash, right side, showing distribution of tracheae,  $\times 44$

dorsal side of the tracheal trunk on each side close to the spiracular branches is a stout branch which runs dorsad for a short distance, and then divides into two secondary branches. One of these (fig. 2, A, *h*) runs dorsad along the line of separation from the segment next preceding and supplies the pericardial cells of this region. The other secondary branch (*i*) takes an oblique course along the suture separating the prescutum and the scutoscuteum, and enters the pericardial cavity in the middle of the segment opposite the valves of the heart in the pericardial intersegmental sinus. Here the branch breaks up into a few tracheoles which are distributed to the walls of the heart. The dorsal branches just described may be designated as "superficial," in contradistinction to other ("deep") branches which supply the viscera. They are branches of considerable size springing from the dorsal side of the tracheal trunks in the intervals between the spiracular branches. In addition to these, each of the superficial branches usually gives off near its base a twig to the viscera. The viscera in

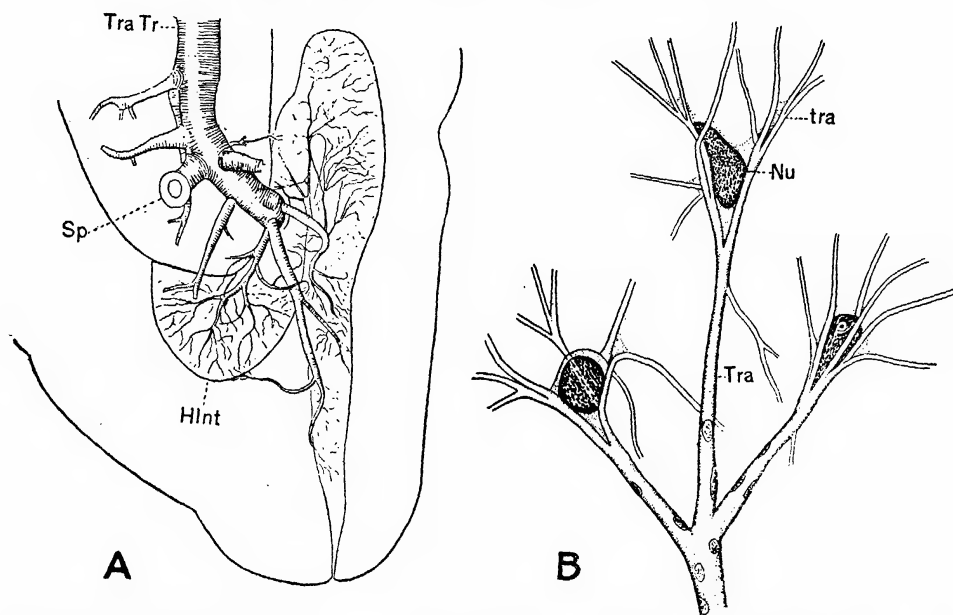


FIG. 3.—A, posterior end of a honeybee larva treated with caustic potash, seen from right side, showing distribution of tracheae to hind-intestine (*HInt*),  $\times$  ca 33; B, distal end of tracheal branch (*Tra*) showing tracheoles (*tra*) and nuclei of tracheal end cells (*Nu*),  $\times$  195

the lower half of their segments are supplied by small twigs given off by the transverse commissures and not shown in the figures. In the 9th abdominal segment a superficial branch is given off caudad of the last pair of stigmata, while from the two ends of the posterior transverse commissure two or three deep branches are given off which supply the hind-intestine, as shown in Figure 3, A.

As in all insects, the tracheae are simple in structure, being merely thin-walled tubes composed of small flat epithelial (pavement) cells and lined with a chitinous intima which is thickened in the form of fine transverse spirally-wound threads, the taenidia. The size of the taenidia is at least approximately proportional to the size of the trachea of which they form a part, while the size of the tracheal cells themselves remains nearly constant in the same individual. Taenidia are found in the tracheal branches up to their termination in tracheal end cells. The structure of the spiracular branches, however, differs somewhat from that of the tracheae proper. At each point where the hypodermal epithelium turns inward to become the wall of a spiracular branch it forms a sharp fold, thus somewhat reducing the mouth of the aperture (Pl. 3, D). The chitinous cuticle also folds

upon itself at every such point, the fold extending toward the middle of the opening in such a way that only a small circular aperture remains. The cells of the wall of the spiracular branch next to the hypodermis, although long-prismatic in form, are of the same size as the latter, and are also covered internally by a smooth layer of cuticle. A short distance within, however, the internal surface of the wall becomes raised up into annular ridges, thus again reducing the diameter of the lumen of the branch so that a distinct antechamber is made, more or less spheroidal in form, bounded externally by the chitinous fold bordering the spiracular opening, and internally by the ridges just mentioned. The section or zone of the branch bearing the ridges comprises about a half of the total length of the branch. The cells are here much larger than elsewhere and usually much vacuolated, particularly at their bases. The ridges are here formed not so much by thickenings of the cuticle as by the contour of the inner ends of the cells themselves, except toward the inner end of this zone, where a number of coarse taenidia are found which in sections present a more or less pinnatifid form. These intergrade with the taenidia of the usual type which are found farther centrad within the branch. Centrad the zone of large cells rather suddenly gives place to the flat pavement type of cells common to tracheae in general. The description just given applies to the larva of intermediate size, about three days old. In mature larvae the zone of large cells is even more sharply differentiated, but the cells no longer combine to form annular ridges on their inner surface. They are very much vacuolated at their bases, and this portion of the spiracular branch presents the appearance of having contracted, reducing the lumen to little more than a cleft (fig. 3, B). The significance of this reduction of the lumen is not evident. The general aspect and the arborescent mode of branching of the tracheae is shown in Figure 3, A. At their tips the branches break up rather suddenly into a tuft of delicate filaments, the tracheoles. At the point of origin of the tracheoles and surrounded by them is a nucleus, of large size as compared with those of the tracheae and of somewhat irregular form (fig. 3, B, *Nu*). Its chromatin is abundant and distributed in the form of coarse granules of fairly uniform size. Surrounding the nucleus is an exceedingly delicate layer of cytoplasm, which accompanies each tracheole and in fact forms part of its wall. This is most clearly seen in transverse sections of tracheoles. The intima, however, is that which gives to the tracheoles their sharp contour, and since they are still visible in caustic potash preparations it is to be inferred that this intima is chitinous. Near their point of origin the tracheoles frequently branch; farther on in their course branching becomes infrequent. The tracheoles of course vary in length. In a preparation of the muscular layer of the mid-intestine of a mature larva a single tracheole was traced for a distance of  $0.548 \pm$  mm. In the pericardial cavity the tracheoles are much shorter than this (about  $0.300$  mm.). After the preliminary branching a tracheole is quite uniform in diameter, and decreases gradually until the double contour is lost, the tracheole finally terminating in a slender point. The course taken by a tracheole differs in accordance with the structure of the organ or tissue supplied by it. For example, on the outer surface of the mid-intestine, which is very richly supplied with tracheoles, the latter run in straight lines or in gentle curves, the majority being parallel with the circular fibers. In the fat body, on the other hand, they pursue a sinuous course, winding in and out between the fat cells.

The relation of the tracheoles to the organs and tissues of the honeybee larva is essentially the same in every case, since nowhere has a tracheole been seen to penetrate a cell, the statements of Leydig (32), Kupffer (30), Lidth de Juede (33), Gilson (12), and Holmgren (16) to the contrary notwithstanding. In the case of the alimentary canal and the heart the tracheoles simply spread out over

the external surface. On the other hand, the brain, ventral cord, and ovaries are not only penetrated by the tracheoles but not infrequently by the finer tracheal branches also, the latter terminating in end cells, which therefore lie within the tissues of the nervous system or of the ovaries, as the case may be. Anastomosis of the tracheoles to form a meshwork, as reported by Wielowieyski (51), Von Wistinghausen (54), Petersen (41), Williams (53), and others was not observed.

The tracheal system of the bee larva offers nothing of special interest in its general form and relations and conforms to the type usual in the higher insects. As compared with the imago the tracheal system is simpler and more generalized. In the abdomen of the imago, however, particularly in segments 3 to 7, the tracheal trunks and their metamerically arranged commissures and branches may easily be recognized, although now enlarged to form air sacs (46). The posterior commissure is wanting. In the thorax the tracheal system is more greatly modified, and the larval type correspondingly obscured.

## ALIMENTARY CANAL, MALPIGHIAN TUBULES, AND SILK GLANDS

### ALIMENTARY CANAL

The alimentary canal comprises a short and slender fore-intestine (Pl. 4, A, *Oe*), a voluminous mid-intestine (*MInt*) and a relatively short hind-intestine, having a sigmoid flexure (*HInt*).

The fore-intestine shows the usual differentiation into three subdivisions, mouth, pharynx, and oesophagus.

The mouth (Pl. 2, B, *Mth*) is a narrow transverse slit bounded above by the clypeus, below by the labium, and laterally by the mandibles. It opens immediately into the pharynx. This is a somewhat ill-defined region, comprising the anterior end of the fore-intestine and extending caudad to about the point where the crura cerebri cross the fore-intestine. The epithelium of the mouth and pharynx (with the exception of the roof of the latter) is precisely similar to the hypodermis of adjacent parts of the head, being composed of closely crowded prismatic cells with flat external ends (Pl. 2, B, and 4, C). On the roof of the pharynx is a conspicuous and well developed epipharynx (Pl. 3, A, and 4, C, *Ephy*), which extends from the mouth well back into the oesophagus. It is broad and flat at its anterior end (Pl. 3, A, *Ephy*), scarcely rising above the level of the adjacent epithelium of the roof of the mouth, but in the pharyngeal region it becomes a highly convex fold of the dorsal wall, projecting ventrad into the lumen of the pharynx and giving the latter a crescentic outline in transverse section (Pl. 4, C, *Ephy*). The cells constituting this fold are very different from those of the remainder of the pharyngeal wall, being of small size and elongated, pyriform or club-shaped in form, their larger ends directed toward the lumen of the oesophagus. On the dorsal surface of the pharynx the cavity of the epipharyngeal fold is crossed by a large number of muscle fibers which unite the two lateral edges of the fold. These are shown in Plate 4, C, and in section in Plate 3, A, *EphyMcl*. Beneath these are a few longitudinal muscle fibers.

On the floor of the pharynx, at its external end, is a small papillate elevation, provisionally labeled the hypopharynx (Pl. 3, A, *Hyphy*).

In addition to the muscles of the epipharynx, the pharynx is provided with other muscles, which are described in this paragraph. In the clypeus a pair of small muscles take their origin from the dorsal wall and are inserted on the epipharyngeal fold close to the median plane. Some four or five pairs of similar muscles are ranged behind one another, having their origin on the dorsal wall of the labrum. These are the levators of the epipharynx (Pl. 4, C, and 6, C, *LevEphy*). Their action is evidently directly opposed to those crossing the

epipharyngeal fold, which, by drawing together the two edges of the fold, force the epipharynx down into the lumen of the pharynx and thus occlude it, while the levators, on the other hand, virtually serve as dilators. A larger pair of muscles having their origin on the dorsal wall of the labrum are inserted on the oesophagus at the lateral margins of the epipharynx, the levators of the pharynx (Pl. 4, C, *LevPhy*). Just ectad of these are inserted still another pair of muscles which are attached to the cranial wall on each side of the base of the labrum, the superior dilators of the pharynx (Pl. 4, C, *SDilPhy*). On the ventral side of the pharynx is a group of muscles having their origin on the anterior face of the central body of the tentorium, which are inserted on the ventral wall of the pharynx. These are the inferior dilators of the pharynx (Pl. 3, A, *DilPhy*). This list comprises the total equipment of muscles possessed by the pharynx.

The pharynx passes without material change in caliber into the oesophagus. This is a cylindrical tube, its walls continuous with those of the pharynx, but formed of cells of a somewhat different character, these being much less crowded than those of the pharynx and varying in form from cuboid to long columnar. Their central ends are rounded instead of flat. This epithelial layer, however, does not always form a true hollow cylinder, being generally more or less infolded at the sides, top and bottom, and the cells being higher at these points, so that the lumen of the oesophagus is frequently quadrangular in section (Pl. 2 A, *Oe*), or even in some cases rosette-shaped. A chitinous cuticle, continuous with that covering the external surface, lines the oesophagus as well as the mouth and pharynx. In the anterior part of the fore-intestine this corresponds in thickness with the external cuticle, but toward the posterior end of the fore-intestine it becomes thinner, and in sections has a much wrinkled and torn appearance. It usually extends a short distance into the lumen of the mid-intestine, forming the "funnel" (Trichter) of Schneider (45). On its external surface the oesophagus is clothed with a single layer of circular (transverse) muscle fibers. Longitudinal muscle fibers appear to be virtually absent. The caudal end of the oesophagus, where it joins the anterior end of the mid-intestine, is reflected back on itself to form an oesophageal valve (Pl. 3, A and 5, C, *OeVlv*) similar to that found in many other insects. The cells of that part of the valve which is reflected back are notable for their attenuated, almost linear form. Muscle cells are lacking between the two folds of the valve.

The mid-intestine (Pl. 4, A, *MInt*) has the form of a long hollow cylinder with rounded ends, and in mature larvae has a diameter about one-third of that of the body. It extends from the prothorax to the ninth abdominal segment. It is hardly necessary to state that structurally the mid-intestine is a blind sac, the posterior end being completely closed. It is anchored in place by numerous tracheae which extend mesiad from the longitudinal trunks. The finer branches of these tracheae terminate in tracheal end cells upon reaching the surface of the mid-intestine. From these end cells numerous tracheoles are sent out enveloping the external surface of the mid-intestine. If the muscular coat of the intestine be stripped off, as can be done in favorable cases, its surface is seen to be closely and uniformly dotted with tracheal end cells, one of which is represented in Plate 5, D, *TraECl*.

The walls of the mid-intestine are composed of a thick single-layered epithelium covered externally by a delicate meshwork of muscle fibers. The cells composing the epithelium (with the exception of a few cells around the anterior end, next to the oesophageal valve) are all alike, being relatively large and having in general a short and rather cubical form, their mesial margins, however, being slightly convex (Pl. 5, B). Here and there are to be seen cells which are pyriform, with their larger ends directed centrad and projecting into the lumen.



There is, however, no evidence that cells or parts of cells of the epithelium are set free in the lumen, as described by Snodgrass (46) for the imago. The cytoplasm of the cells of the mid-intestine is always deeply stained in sections (Pl. 5, A, *MInt*) and has the appearance of being traversed by numerous parallel fibrillae which run at right angles to the surface of the epithelium. A similar appearance is found in the secreting cells of the silk gland in certain Lepidoptera (12) and in the epithelial cells of the mid-intestine of Ptychoptera (11). The nuclei are spherical, as a rule, and contain a large number of chromatin granules. In fixed material, at least, these granules, along with the achromatic material, are agglomerated into a dense spherical mass within the nuclear membrane, leaving a peripheral space vacant. This condition may, however, be an artifact. On their mesial surfaces the cells of the mid-intestinal epithelium show a well-developed striated border (Pl. 5, B, *StrBor*) similar to that observed in Ptychoptera (11) and other insects. Wedged in between the bases of the cells, next to the basal membrane (intima) triangular groups of minute cells are seen here and there. These are the imaginal cells of the mid-intestine (Pl. 5, B, and C, *ImCls*).

The mid-intestine is lined throughout by a homogeneous layer of unknown chemical nature, apparently of gelatinous consistency, and having a thickness two or three times that of the epithelium, the so-called "peritrophic membrane" (Pl. 5, C, *Pmb*). In sections it is more or less finely granular in structure. Peripherally it is in intimate contact with the epithelium of the intestine; centrally it is sharply demarcated from the enclosed food mass. Not a little has been written concerning this somewhat problematical structure in various insects, but a full review of the literature will not be attempted here. Rengel (44) has described it in the larvae of the wasp (*Vespa*) and honeybee, Strindberg (47) in the ant embryo, and Snodgrass (46) and Petersen (41) in the adult honeybee. Rengel states that in larvae the peritrophic membrane has a laminated structure consisting of several concentrically arranged membranes, as is plainly the case in the adult, but in all of the writer's preparations of this membrane he has never observed this structure except at the anterior end of the mid-intestine. Elsewhere the membrane is thick and homogeneous, but here the epithelial cells grow rapidly and progressively smaller, forming a ring around the oesophageal valve (Pl. 5, C, *x*) and are separated from the latter by a narrow space. These cells also differ from those of the remainder of the mid-intestine not only in being of smaller size but also in lacking, at least to a large extent, the striated appearance of the cytoplasm. From the surface of these cells thin streams of secretion can plainly be seen running caudad and joining with the principal mass of the peritrophic membrane within the mid-intestine. Streams of this material also appear leading inward from the three rows of epithelial cells next to the ring of smaller cells, as shown in Plate 5, C, and this appearance is characteristic of all of the sagittal sections examined, including preparations of both young and old larvae. It suggests that the cells of the anterior rim and those of the two or three adjacent rows are responsible for the secretion of the peritrophic membrane; an interpretation precisely in line with the view advanced by Van Gehuchten (11) in the case of the dipterous larva Ptychoptera and Cuénot (8) in Orthoptera. Strindberg, however, has advanced against this view the fact that in the ant larva peritrophic membrane is continuous over the closed posterior end of the mid-intestine, which, he states, could not be the case if the membrane were secreted by an anterior rim of cells. This objection, however, would have less weight if, as seems probable, the peritrophic membrane is fluid or semi-fluid, since from its nature it would tend to form a uniform layer over the area of contact and therefore would flow together over the posterior end. It should be noted that the peritrophic membrane described above has nothing whatever to do with the cuticle secreted by the oesophagus, the free

posterior ends of which frequently project far into the anterior end of the mid-intestine (Pl. 5, C, *Cu*).

The external surface of the mid-intestine, as already stated, is covered by a delicate network of muscle fibers. A small portion of this sheet, stripped off from the mid-intestine, is shown in Plate 5, D. The central or inner surface is uppermost. The inner layer (*LMcl*) is composed of branched muscle cells whose delicate processes, connecting adjacent cells and thus forming an anastomosing plexus, tend to run in a longitudinal direction. Below these, and morphologically outside of them, is a layer of transverse muscle fibers (*TMcl*). These are closely and uniformly spaced, and are also quite uniform in caliber. The nuclei of the cells composing this layer are much smaller than those of the inner layer. The muscle cells of the transverse layer are also interconnected by extremely delicate protoplasmic processes, some of which are indicated in the figure.

The hind-intestine (Pl. 4, A, *HInt*) is a cylindrical tube of fairly uniform diameter except at its anterior end, where it shows a funnel-like or bell-shaped expansion, the larger end of which is applied to the posterior end of the mid-intestine. From this point it bends ventrad, taking a cephalad course to the sixth abdominal segment, where it bends sharply upon itself and takes a direct course caudad to its termination on the tenth abdominal segment. The hind-intestine of the honeybee larva exhibits a simpler structure than that of the ant larva, in which the posterior portion of the hind-intestine is enlarged, narrowing again near the anus, three divisions being thus formed, small intestine, large intestine and rectum (35). This condition also exists in the larva of the beetle *Anobium* (23).

As already stated, the anterior end of the hind-intestine exhibits a funnel-like or bell-like enlargement (Pl. 4, A). The end of the funnel is, however, not open, but is closed by an extremely thin layer of minute cells (Pl. 6, A, *dph*), which is continuous around the margin of the funnel with the epithelial wall of the mid-intestine. This epithelium, on the sides of the funnel-like enlarged portion, is thick and composed of a large number of slender club-shaped cells (Pl. 6, A, *HInt*). These give place, at the neck of the funnel, to larger prismatic or columnar cells. The remainder of the epithelium of the hind-intestine is formed of cells of this type. This is thrown into deep folds, as shown in Plate 5, E, projecting almost to the center of the lumen and giving the latter a stellate aspect in section. Relatively stout muscle fibers (*TMcl*) encircle the hind-intestine, being found from the margin of the funnel-like expansion to the anus. These are for the most part arranged in a single layer, but toward the posterior end they become more numerous and are arranged in two or even three layers. Outside of the transverse muscles are a few irregularly arranged longitudinal fibers (Pl. 5, E, *LMcl*). In the 10th segment numerous slender muscle fibers run between the lateral hypodermis and the lateral walls of the hind-intestine.

The posterior termination of the hind-intestine, the anus, is a simple transverse slit situated on a slight elevation in the center of the 10th abdominal segment.

The mode of attachment of the mid- and hind-intestines in the larvae of the aculeate Hymenoptera is of special interest and has been studied and described in detail by Rengel (44) in the wasp (*Vespa*), in the ant (*Lasius*) and in the honeybee. With the exception of minor details, it is the same in the three groups. As shown in Plate 6, A, at the extreme posterior end or fundus of the mid-intestine there is an outpushing (*st*) of the epithelium, thus forming a rounded projection on the external surface of the fundus. In *Vespa* this projection is quite prominent, forming the "stalk" (*Stiel*) of Rengel. In the honeybee larva it is externally merely a low rounded eminence; internally it is represented by a depression of the epithelium, the bottom of which is formed by a heap of irregular transparent cells,

smaller than those of the adjacent epithelium. The muscular investment of the mid-intestine (*MclEnt*) ceases abruptly at the margin of the "stalk." As already stated, the enlarged anterior end of the hind-intestine is closed by a thin layer of minute cells, forming a circular structure (*dph*) which may conveniently be termed "the diaphragm." The central part of the diaphragm is closely adherent to the stalk, as described by Rengel. In larvae of *Vespa* and of ants the diaphragm is relatively much thicker than in those of the honeybee, judging from the figures given by Rengel (44), Karavaev (22) and Pérez (40), but otherwise the relations are essentially similar.

#### MALPIGHIAN TUBULES

The Malpighian tubules are four in number and lie, two on each side, between the lateral faces of the mid-intestine and the body wall (Pl. 4, A and 5, A, *Mal*). They pursue a winding course, being thrown into numerous loops and folds, and extend from about the last thoracic to the 9th abdominal segment. In the newly hatched larva the tubules are slender and of nearly uniform diameter throughout; in the mature larva their anterior ends only are slender, the tubules widening gradually caudad, reaching their maximum diameter, which is about one-half that of the mid-intestine, in the region of the 7th abdominal segment (Pl. 4, A, *Mal*). From this point they taper rapidly to their caudal (or central) ends, which are inserted, two on each side, between the mid-intestine and hind-intestine where the two latter have their junction. These central ends are exceedingly slender and pointed, composed of relatively small cells, and become closely attached to the thin epithelium closing the anterior end of the hind-intestine, with their tips abutting on the stalk of the hind-intestine (Pl. 6, A, *Mal*). The lumen of the Malpighian tubules becomes greatly reduced here and finally ceases, the central ends of the tubules therefore ending blindly, contrary to the statements commonly found in the literature on this subject (44). The writer (36) stated that in the newly hatched larva the Malpighian tubules open into the mid-intestine, but renewed investigation shows that there is no basis for the statement, since in all the sections examined, both cross and sagittal, the central ends of the tubules disappear in the relatively thick layer of cells closing the anterior end of the hind-intestine. Sections of a larva two days old also fail to disclose any connection between the lumen of the Malpighian tubules and that of the hind-intestine.

The walls of the Malpighian tubules consist of a single layer of epithelial cells. In very young larvae the wall of the tubules is relatively thick and the centrally placed lumen correspondingly small. In mature larvae the cells composing the walls are flat, thin, and pavement-like in character (Pl. 5, A, *Mal*, and Pl. 4, B and D). This condition exists even in the slender anterior ends, clearly indicating that the tubules are distended by internal pressure. The nuclei are discoid (Pl. 4, D, *Nu*), being flattened in a plane normal to the surfaces of the tubules, and contain abundant chromatin in the form of subequal spherules. The cytoplasm of the cells displays no fibrillae, as in the case of the cells of the mid-intestine and silk glands, and appears to be finely granular. The margins of the cells at the point of junction with one another are uniformly vacuolated, as shown in Plate 4, D, thus giving the tubules, stained or unstained, a characteristic mottled appearance in surface view (Pl. 4, B). The tubules are lined within by a cuticular intima, possibly chitinous, which stains much less densely than the cytoplasm (Pl. 4, D, *Int*).

The condition just described is characteristic of larvae shortly before the capping of the wax cell. Younger larvae show intermediate conditions, that is, the diameter of the tubules is relatively smaller and the walls thicker. The

next phase in their development takes place subsequent to capping and is directly correlated with the establishment of communication between the lumina of the mid- and hind-intestines, and the consequent evacuation of the feces. This takes place some time during the twenty-four hours subsequent to capping, and has been treated at length by Rengel (44). In brief it consists, as shown in Plate 6, A and B, in an outpushing (caudad) of the fundus of the mid-intestine, that is, of the stalk, into the lumen of the anterior enlarged end of the hind-intestine and the subsequent perforation of the stalk and also of the diaphragm to form a tubular opening through which the fecal accumulations of the mid-intestine are forced by contractions of the latter. This mass of feces is omitted in Plate 6, B. In larvae of this stage the central ends of the Malpighian tubules no longer end blindly, but each now opens into the hind-intestine by a minute pore-like aperture. These openings are close to the junction of the mid- and hind-intestines, as shown on the left side in Plate 6, B, *Mal*. The appearance of this and other preparations suggests that the central ends of the tubules have perforated the diaphragm while at the same time their lumina became extended to communicate with that of the hind-intestine. A curious feature of the efferent ducts of the Malpighian tubules of this stage is the appearance of structures which in fixed preparations resemble motile cilia. That the Malpighian tubules are now emptying themselves of their contents is strikingly shown by sections of the tubules at points more or less remote from the place of attachment. Whereas in sections of mature larvae prior to the establishment of communication between the mid- and hind-intestines the Malpighian tubules have in general the appearance of thin-walled tubular sacs, they now show a much diminished caliber and the cells forming the walls are no longer flat, but are cubical in form.

The accumulation of solid excreta in the blind mid-intestine of the bee larva is therefore paralleled by the accumulation of fluid excreta (urates?) in the blind Malpighian tubules. Both discharge their accumulations at the same time. The advantage of this in the life history of the larvae is obvious.

The imaginal Malpighian tubules appear just previous to capping (Pl. 6, A and B, *mal*), as outgrowths of the anterior enlarged section of the hind-intestine (1).

#### SILK GLANDS

The silk glands comprise a pair of slender cylindrical tubules, thrown into numerous short convolutions, and extending from the posterior border of the mesothoracic segment to the neighborhood of the sixth abdominal segment (Pl. 4, A, *SilkGl*). Their location, relative to the other organs, is shown by Plate 5, A, *SilkGl*. They lie, one on each side of the mid-line, closely surrounded by fat cells, about halfway between the mid-intestine and the ventral body wall. At about the posterior margin of the mesothoracic segment the glands, strictly speaking, terminate, and each gland here opens cephalad into a thin-walled duct which pursues a straight course into the head, passing close to the ventral wall and beneath the suboesophageal ganglion, where it meets its mate from the opposite side. The two ducts unite to form a common duct (Pl. 2, A, *SilkD*) which terminates on the tip of the labrum. The common duct is cylindrical with the exception of its anterior end, where it rather suddenly widens out laterally and opens by a narrow transverse slit on an elevation, also transversely elongated, situated on the tip of the labrum (Pl. 1, F, and 3, A, *SilkDO*).

The finer structure of the silk glands is illustrated by Plate 5, F and G. Plate 5, F, represents an oblique transverse section through one of the silk glands near its posterior end. The cells forming the wall of the tube are in many respects similar to those forming the mid-intestinal epithelium, although much smaller, the nuclei of the walls of the silk gland having a diameter scarcely more than a

third of that of the nuclei of the mid-intestinal epithelium. The cells forming the walls of the silk gland are like those of the mid-intestine in the deeply-staining character of their cytoplasm, which usually stains so densely in comparison with the other tissues as to be almost totally opaque, as shown in Plate 5, A, *SilkGl*. Other points of similarity are the form of the cells, whose breadth and height are approximately equal, the striated or fibrillated aspect of the cytoplasm, noted also in the cells of the silk gland of Lepidoptera and Trichoptera (12), and the spherical nuclei, each containing a compact mass of chromatin granules. Near their anterior ends the character of the cells forming the wall changes somewhat. Here the walls are thicker, the cells composing them being smaller and long-columnar in form. The cytoplasm stains less deeply and no longer shows well-marked fibrillae. The lumen of this portion of the gland shows within it coagulated secretion, although the larva in question was scarcely mature.

The ducts of the silk glands are thin-walled and otherwise histologically precisely similar to the tracheae, this similarity extending to the possession of a chitinous intima, thickened spirally to form taenidia.

## MUSCULAR SYSTEM

### MUSCLES OF THE HEAD

The muscles of the bee larva may be divided for descriptive purposes into muscles of the head and muscles of the trunk, since these two sets are totally different. The principal muscles of the head are as follows:

#### MANDIBLES

Each of these is provided with an extensor and a flexor muscle. The extensor (Pl. 6, C and D, *EMd*) is inserted on the inner surface of a papillate elevation of the hypodermis located on the ectal side of the base of the mandible. From this point it passes directly caudad to its origin on the fold of hypodermis separating the head and trunk (neck fold) close to the outer end of the transverse arm of the tentorium. The flexor muscle (Pl. 6, C and D, *RMd*), which is the largest in the head, is inserted on a long and pointed hollow spine, the mandibular apodeme (Pl. 1, A, and 6, D, *RAp*), which arises from the mesial side of the base of the mandible (*Md*). From this a stout bundle of muscle fibers passes caudad parallel to the fibers of the extensor muscle to an origin just dorsad of them. Another and larger group of fibers breaks up into small subequal bundles, passing behind the brain. These are attached at regular intervals along the neck fold between the point of origin of the extensor muscle and the mid-dorsal line of the head (Pl. 6, C, *RMd*).

#### MAXILLAE

Mesial and dorsad of the base of each maxilla, below the apodeme for the extensor of the mandible, there is a papillate elevation of the hypodermis, on the inner surface of which is inserted the flexor muscle of the maxilla. This is of small size (Pl. 6, C, *RMx*), and originates on the neck fold, just ventrad of the origin of the extensor muscle of the mandible. The maxillae have no extensor muscles corresponding morphologically to those of the mandibles. They are, however, provided with a pair of strong muscles which appear to be functionally extensor muscles. Each of these takes its origin as a broad band from the ventral surface of the anterior arm of the tentorium of the corresponding side, close to the posterior end of the arm, and from here passes ectad and ventrad

to an insertion on the ventro-lateral surface of the head, just caudad of the base of the maxilla (Pl. 6, C, *EMx*). It apparently serves to rotate the maxilla outward.

#### LABIUM

The labium is provided with two pairs of retractors. The larger or major retractors (Pl. 6, C, *IRLb*) take their origin from the neck fold just ventrad of the external ends of the transverse arms of the tentorium. From there they run cephalad, converging meanwhile, and are inserted, a short distance apart on either side of the ventral mid-line, on the posterior margin of the labium. These muscles by their contraction withdraw the labium into the ventral part of the head. The minor retractors (Pl. 6, C, *2RLb*) are slender muscles which have their origin directly cephalad of the insertions of the major retractors. From here they run cephalad, converging at the same angle as the major retractors, to the tip of the labium where they are inserted on the wall of the common duct of the silk gland. These muscles serve to retract the tip of the labium. The two sets of retractors, when seen from the ventral surface, form a figure resembling the letter V, inverted.

#### LABRUM

At the base of the clypeus is a pair of well defined muscles (Pl. 2, B, and 6, C, *ClpMcl*), each of which takes its origin from the head capsule at the dorsal margin of the line of junction with the anterior arms of the tentorium. From these points the two muscles pass dorsad and slightly mesiad to insertions on minute apodemes on the dorsal wall of the head capsule, near the mid-line, at the base of the clypeus. These insertions are marked on the exterior by shallow depressions of the surface (see Pl. 1, F). Immediately cephalad of the insertions of these muscles, a second pair of muscles, the retractors of the labrum (Pl. 3, A, and 6, C, *RLm*), have their origin. These are inserted on the posterior edge of the clypeus in the dorsal mid-line.

Certain other muscles located in the labrum and clypeus and associated with the pharynx are described in the section on the alimentary canal (see p. 1184).

#### CRANIAL MUSCLES

Two other pairs of muscles are found in the head which pass from the tentorium to the cranial wall. The muscles of the first pair are slender, and originate on the dorsal surface of the anterior arms of the tentorium, near the junction of the latter with the transverse bar. These two muscles then pass dorsad on either side of the oesophagus, between the two halves of the brain, and are inserted on the median fold of the dorsal cranial wall. It is worthy of note that in preparations of larvae which have completed their growth these muscles appear to have lost their attachment to the dorsal cranial wall. This change is presumably related in some way to the approaching metamorphosis. The muscles of the second pair are also well developed in young larvae, but insignificant in older ones. Each of these muscles takes its origin from a long spur on the dorsolateral face of each of the anterior arms of the tentorium (Pl. 1, A, *ApTen*). These spurs are directed dorsad and laterad. In the young larvae the muscle fibers are directed dorsad and laterad, diverging meanwhile, and have broad insertions on the lateral walls of the head capsule, laterad of the cerebral lobes (36). In old larvae the spurs are long and directed cephalad and dorsad, extending nearly to the anterior cranial wall (Pl. 6, D), the short gap being bridged by a few muscle fibers inserted on the anterior wall of the cranium just dorsad of the antennal rudiments. The points of insertion are marked externally by well-defined circular depressions (see Pl. 1, F). Kirmayer (26) finds similar spurs on the tentorium and corresponding muscles in the head of the *Vespa* larva.

## MUSCLES OF THE TRUNK

The muscles of the trunk have a strictly metameric arrangement and conform more or less closely to a definite and typical arrangement in all segments. The arrangement of the muscles in the thorax differs slightly from that of the abdominal muscles. In the abdomen the same arrangement of muscles prevails in all the segments with the exception of the 10th. In the 10th segment the muscles characteristic of the other segments are almost entirely wanting, the only

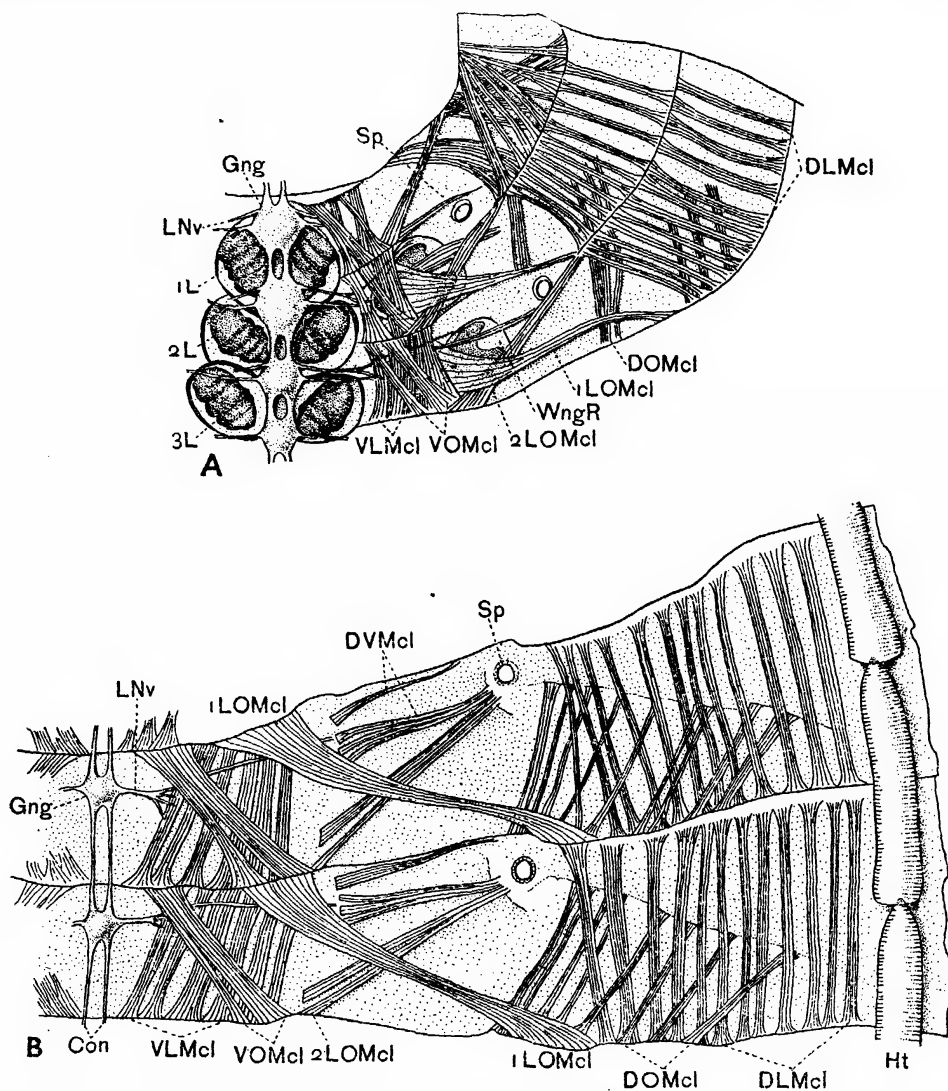


FIG. 4.—A, inner surface of right half of body wall of thoracic segments, showing musculature,  $\times 10$ ; B, inner surface of right half of body wall of fourth and fifth abdominal segments, showing musculature,  $\times 10$

trunk muscles present being a few fibers running from the lateral border of the anus to the anterior (lateral) borders of the segment.

The trunk muscles, taking the 4th and 5th abdominal segments (fig. 4, B) as typical, are the ventral longitudinal, ventral oblique, lateral oblique, dorso-ventral, dorsal longitudinal, and dorsal oblique.

## VENTRAL LONGITUDINAL MUSCLES

These muscles (fig. 4, B, *VLMcl*) are not, strictly speaking, longitudinal, since they diverge slightly from the long axis of the larva in a cephalo-laterad direction.

The total effect of their action is, however, to contract the ventral surface in a longitudinal direction. They are flat bands, 10 or 12 in number, disposed on either side of the ventral mid-line, close to the hypodermis, and extend between the anterior and posterior margins of each segment.

#### VENTRAL OBLIQUE MUSCLES

These (*VOMcl*) overlie the ventral longitudinal muscles, crossing them at an angle of about 45 degrees and running in a cephalo-mesiad direction, also connecting the anterior and posterior borders of the segment. Each consists at its anterior end of a broad band of fibers which splits into two subequal bands at its posterior end.

#### LATERAL OBLIQUE MUSCLES

These include two sets of muscles, which may be designated as major oblique (*1LOMcl*) and minor oblique (*2LOMcl*). The major oblique muscles are of large size and connect the anterior and posterior borders of their respective segments. Their anterior ends are attached to the body wall directly adjacent to the line of attachment of the posterior ends of the ventral oblique muscles of the segment preceding. From this point each of these muscles runs laterad and caudad, spanning the lateral fold of that side, to an attachment on the posterior border of the segment approximately on a line with the spiracles. The minor lateral oblique muscles (*2LOMcl*) comprise two small bands lying close to the body wall under the major oblique muscles. One of these small bands is attached to the posterior margin of the segment just dorsad (ectad) of the posterior attachment of the ventral oblique muscle; the other band is attached to the body wall close in front of this point. These two bands take a dorsad and cephalad course, meanwhile diminishing in breadth to a point on the body wall just ventrad of the spiracle (*Sp*).

#### DORSO-VENTRAL MUSCLES

Two pairs of dorso-ventral muscles (*DVMcl*) are present in each segment. The muscles of the smaller pair run close to the anterior margin of the segment from a point just dorsad (ectad) of the anterior attachment of the major oblique muscle to an attachment on the intersegmental body wall about on a line with the spiracles. The second set of dorso-ventral muscles comprises three small muscle bands on each side of the segment, having their ventral attachment to the body wall just caudad of that of the smaller muscles just described, about one-fourth of the width of the segment from its anterior margin. Their dorsal attachment is close beside that of the minor lateral oblique muscles, below the spiracles.

#### DORSAL LONGITUDINAL MUSCLES

These (*DLMcl*) comprise a set of narrow bands, from 10 to 12 on each side, underlying the dorsal body wall and divided by the heart into two bilateral groups. Their arrangement is truly longitudinal and the area occupied by them extends ventrad on each side almost as far as the spiracles. These muscles connect the anterior and posterior margins of the segments.

#### DORSAL OBLIQUE MUSCLES

The dorsal oblique muscles (*DOMcl*) include a row of from 10 to 12 slender bands on each side. Their posterior ends are attached to the posterior margin of the segment, the line of attachment extending dorsad from a point about midway between the dorsal and ventral mid-lines halfway to the dorsal mid-line. From here these muscles run cephalad and dorsad, diverging slightly, and are attached



to the dorsal body wall of each side along the suture separating the prescutum from the scuto-scutellum and in fact determining this sutural line.

In the thoracic region (fig. 4, A) the musculature is slightly different from that of the abdomen. The ventral longitudinal and oblique muscles (*VLMcl*, *VOMcl*) are readily recognized, although here their lateral extent is reduced, especially in the prothorax. The dorsal longitudinal muscles (*DLMcl*) are present in all three thoracic segments. In the prothorax there are present two groups of dorsal oblique muscles whose arrangement may be gathered from the figure. In the mesothorax and metathorax the dorsal oblique muscles are divided into two parallel groups. A strong muscle (*2LOMcl*) crosses the lateral face of each of the three segments, running from its posterior border cephalad and dorsad to its anterior border. This muscle is absent in the abdominal region. In the meso- and metathorax a very large muscle (*1LOMcl*) is attached by a broad line of attachment to the ventral side of the segment, mesiad of the leg rudiments (*1L-3L*), and runs laterad and caudad to the posterior margin of the segment near the lateral margin of the area occupied by the dorsal longitudinal muscles. This muscle evidently corresponds to the major oblique muscles of the abdomen. In all three thoracic segments the minor lateral oblique muscles (*2LOMcl*) connect the anterior and posterior margins of their respective segments, crossing the lateral body wall in an oblique direction. It seems doubtful whether these muscles are truly homologous with the minor lateral oblique muscles of the abdominal segments. In the mesothorax and metathorax a dorso-ventral muscle spans the imaginal wing disks. This muscle is much larger in the prothorax than in the metathorax.

The effects of the contraction of the various trunk muscles may be inferred from their size, direction, and attachments. These effects may readily be seen, however, on the external surface of the larva. If the figure showing the external features of the larva (Pl. 1, D) be compared with figure 4, B, it will readily become evident that the superficial contour of the larva is determined principally, if not exclusively, by the trunk muscles. The evident division of the trunk into segments is not, as in the imago, due to the formation of sclerites, but is produced principally by the longitudinal trunk muscles, aided, of course, by the ventral oblique, the major lateral oblique, and the smaller dorso-ventral muscles. The epipleural lobes are referable to the action of the major oblique muscles, the sharp demarcation of these lobes from the sternal area being apparently due to a certain amount of rigidity imparted to the body wall in this region by the ventral longitudinal and oblique muscles, since the area occupied by these coincides with this area. The depressions in which the spiracles are located are produced by the action of the minor lateral oblique muscles, aided, of course, by the posterior set of dorso-ventral muscles. The sutural lines obliquely traversing the dorso-lateral surface of the segments are plainly to be identified with the anterior insertions of the dorsal oblique muscles.

Detailed accounts of the muscular systems of hymenopterous larvae are lacking. The account by Carrière and Bürger (7) of the muscular system of the mason bee and that by Anglas (1) of the muscular systems of *Vespa* and the honeybee are extremely brief. Both merely recognize dorsal and ventral longitudinal muscles, and a set of obliquely arranged dorso-ventral muscles. An examination of the accounts of the muscular systems of the larvae of other orders, such as that recently given by Forbes (10) for lepidopterous larvae, discloses no basis for comparison with the bee larva. The muscles of the latter are, in comparison, few and simple, as might be inferred from its mode of life.

Anglas (1) states that some of the larval muscles, more particularly those of the abdomen, persist with slight modification through nymphosis into the imago. A study of the muscles of the abdomen of the imago shows them to be so different

in number and arrangement from the trunk muscles of the larva that identification of any given muscle or set of muscles of the imago with those of the larva proves to be impossible without a knowledge of the intervening stages.

The general features of the histology of the larval muscles has already been given (36). This description applies to the newly hatched larva, but no essential changes take place during larval growth. In this account it is stated that the muscles of the young larva are not striped. However this may be, in the old larvae cross-striped muscles are the rule. Mention should be made of the constant occurrence of very distinct fibrillae, "tomomitosomes," Maziarski (34), "filaments de resistance," Janet (20, p. 54), in the hypodermal cells at the points of attachment of muscles. These fibrillae frequently assume a brush-like form, as shown in Plate 6, D, at both of the ends of the dilator of the pharynx and at the posterior ends of the mandibular muscles. The filaments bind muscle, hypodermis, and cuticle firmly together, as shown in fixed material by the clinging of the cuticle, elsewhere loosened, to the hypodermis at the points where muscles are inserted.

## HEART AND BLOOD CELLS

### HEART

The heart of the larva is in most respects like the less differentiated portion of the imaginal heart which is situated in the abdomen. The heart consists essentially of a slender thin-walled tube situated in the mid-line close beneath the dorsal hypodermis. It is widest (about 0.25 mm.) at its posterior end and gradually diminishes in caliber up to the anterior border of the 2d trunk (the mesothoracic) segment. Here the heart leaves the dorsal body wall and bends downward, passing beneath the anterior tracheal loop (Pl. 3, A, Ao), at the same time diminishing rapidly in diameter, and is continued cephalad as the aorta. The aorta is not strictly tubular but is open on the ventral side, having in transverse section the form of an inverted letter U, the free edges hanging down on each side of the oesophagus and becoming clothed on the exterior with a layer of tracheoles. At the posterior side of the brain the aorta becomes reduced in its dorso-ventral diameter to enter the narrow cleft between the oesophagus and the upper ends of the crura cerebri and finally terminates at the anterior face of the brain. The posterior end of the heart terminates blindly in the 9th abdominal segment. In life the heart is transparent, and since it is bounded on either side by the relatively opaque white fat cells, it produces externally the appearance of a dark band along the dorsal mid-line of the larva.

At the middle of trunk segments 2 to 11, inclusive, the heart is sharply constricted and is thus divided into 11 chambers. These constrictions, however, do not affect the dorsal and ventral walls of the heart, but only the lateral walls, which are in fact indented by a series of pairs of opposite V-shaped indentations the open ends of which are directed slightly caudad. At the bottom of each indentation is a linear slit; these slits constitute the ostia (fig. 5, B, Ost). The ostia are not, however, precisely normal to the long axis of the heart, but are slightly oblique, their dorsal ends being slightly caudad of their ventral ends.

The action of the heart is simple and similar to the heart action of many other insects. The heart walls bordering on the ostia form valvelike flaps projecting inward which allow a free inrush of blood during diastole, but which automatically close the ostia during systole. The posterior pair of flaps project inward far enough also to close the posterior ends of the heart chambers during systole, preventing a backward flow of blood. Moreover, the thickened margins of the ostia share in the contractility of the heart wall, so that probably these also possess the ability to contract the ostia and the posterior ends of the heart chambers as well, thus acting as sphincter muscles.

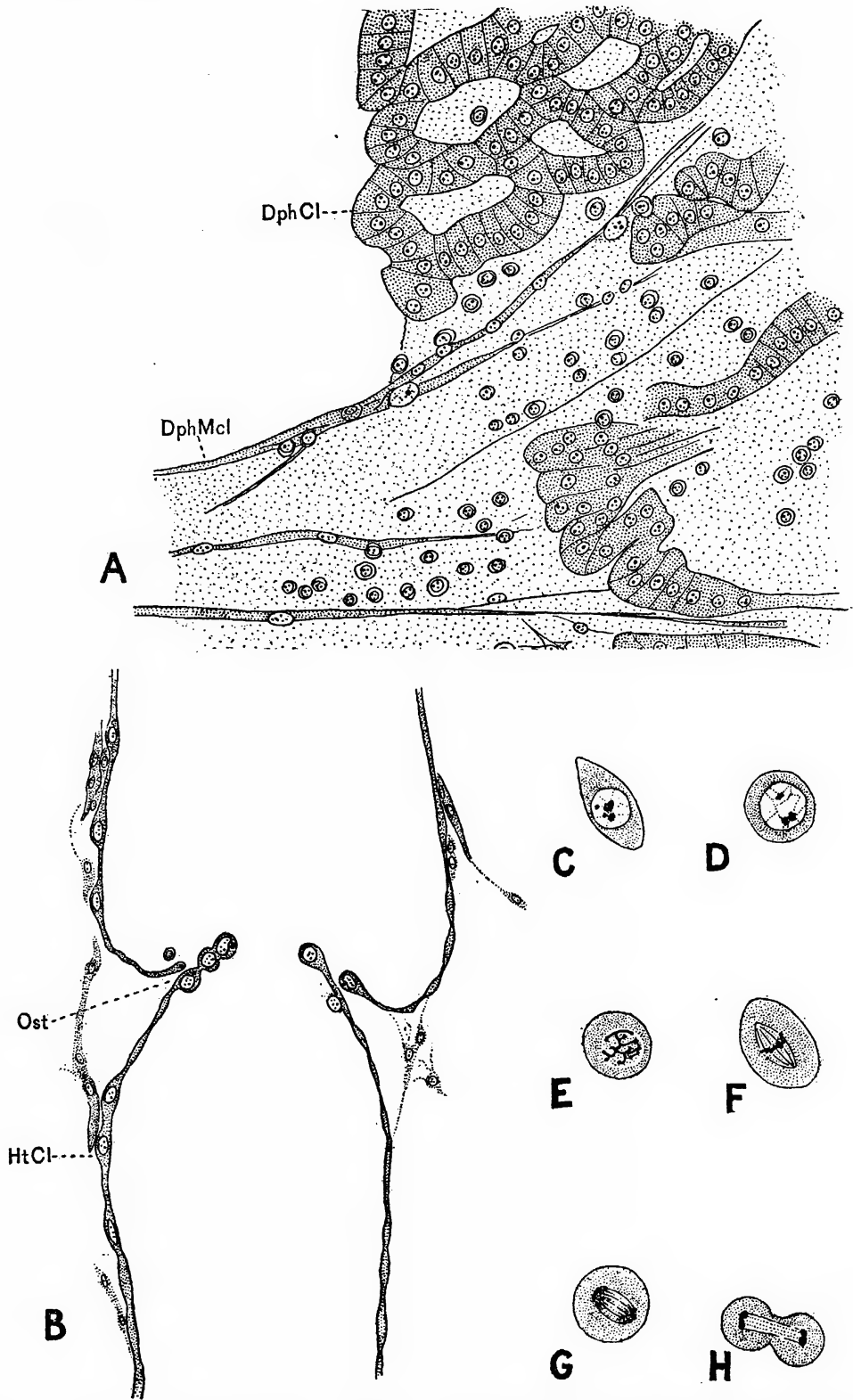


FIG. 5.—A, part of dorsal diaphragm of old larva, showing diaphragm cells (*Dph Cl*) and muscle fibers (*Dph Mcl*),  $\times 66$ ; B, longitudinal (coronal) section of heart in the region of the second thoracic segment, showing ostia (*Ost*) and valves.  $\times 260$ ; C—H, blood cells (lymphocytes),  $\times 600$

The wall of the heart is exceedingly thin, as shown by the figures, and is composed of a double row of cells, corresponding to the cardioblasts of the embryo, the two rows constituting the right and left sides of the heart, respectively. These cells have the form of the half of a radially compressed ring (Pl. 7, B). At the middle of its length each cell is thickened and at this point a large discoid nucleus is found. Traversing each of the heart cells lengthwise, that is, at right angles to the long axis of the heart and mesiad of the nucleus, is a bundle of delicate fibrils, few in number, usually visible only under high magnification. They are most readily seen in cross sections of the heart cells, that is, in longitudinal sections of the heart itself, which have been counter-stained with eosin, the muscle fibrils of the heart cells, like those of other muscles in the larva, readily taking this stain. Their appearance in section is shown in figure 5, B, where they are represented in black. An attempt to represent these in lateral view is made in Plate 6, E, but they are actually less evident than here represented. In suitably stained preparations this bundle of fibers is plainly seen to be transversely striated (Pl. 6, F). The heart cells are therefore muscle cells, essentially similar structurally to other muscle cells in the bee larva, for example the trunk muscles, but in which the fibrillae (myofibrils) are more feebly developed. Similar conditions of structure are found in the cells of the heart wall of the larva of *Tipula* (48) and of those of *Vespa* (1) and *Aeschna* (55). Since the heart of all insects is contractile, it seems probable that further investigation will show such a differentiation to be general. In the larva of the honeybee, the myofibrils may easily escape notice, since larvae stained to show other structures to the best advantage scarcely show them at all. A deep and heavy stain with iron haematoxylin is necessary to reveal the myofibrils in face view; that is, in tangential sections of the heart. The arrangement of the heart cells in a double row is evidently not unique, since in addition to being characteristic of insect embryos it has been described for the *Tipula* larva by Viallanes and for the *Aeschna* larva by Zawarzin (55).

The heart is clothed externally by a loose meshwork of minute branched connective cells, the meshes tending toward elongation in a longitudinal direction (Pl. 6, E and F, *ConCls*). This meshwork serves to strengthen the heart wall and also to anchor the heart in position, since many of the processes of the connective tissue cells extend out to the hypodermis and to the adjacent fat cells.

#### DORSAL DIAPHRAGM

The dorsal diaphragm of the larva is well developed only in the posterior region, from the 4th to the 9th abdominal segments, inclusive, and here it is very similar in structure to that of the imago. In these segments it forms a continuous sheet, attached to the heart on its ventral surface (Pl. 7, A, B, *DDph*) and extending laterad, parallel with the dorsal body wall on either side of the dorsal midline, a distance somewhat less than one-eighth of the entire circumference of the larva. It partitions off a narrow dorsal space, the dorsal sinus or pericardial cavity. The lateral margins of the dorsal diaphragm are free except where they cross the lines of separation of the segments, and here the margins are attached to the body wall. Between these points the membrane is somewhat retracted mesiad, giving the margins a wavy contour (Pl. 7, A). The dorsal diaphragm terminates in the anterior half of the 9th abdominal segment with a free border. Anglas (1) states that the two delicate membranes ensheathing the diaphragm cells are reflected in the median plane below the heart to form a sort of septum which does not reach ventrad to the mid-intestine. This membrane, or septum, which is termed the "cloison médiane," is said to separate the fat body into two symmetrical halves. This account, as regards the bee larva, appears to be incor-

rect in several particulars. The "cloison médiane" is restricted to the anterior segments of the trunk; it does not form a continuous membrane; it does sometimes become attached to the muscular coat of the mid-intestine; it does not divide the fat body, since this is already divided mesially by a space or sinus dorsad to the mid-intestine; and it does not appear to be continuous with the two halves of the dorsal diaphragm, but represents delicate processes of connective tissue cells attached to the ventral surface of the heart. It is apparently absent in mature larvae and is probably a structure of minor importance.

Structurally the dorsal diaphragm consists of two very delicate membranes, having the appearance of basement membranes and possibly being chitinous. These membranes are attached to the ventral wall of the heart in the mid-line. Between the two membranes are situated the diaphragm cells (fig. 5, A, *DphCl*). These are small cells, quadrangular in form, each having a spherical nucleus which occupies the greater portion of the cell. The diaphragm cells are arranged in single rows or strings which are bent in a sinuous form and frequently anastomose, forming a sort of lacework and giving the dorsal diaphragm in surface view a curious vermiculated pattern (Pl. 7, A). These cells are not found anterior to the middle of the fourth abdominal segment. The diaphragm cells are closely covered on their dorsal and ventral faces by the two membranes, which here have the appearance of limiting membranes secreted by the cells (Pl. 7, B, *DDph*). Elsewhere, in the open spaces between the diaphragm cells, the two membranes are generally evident, but are often found in close apposition, giving the appearance of a single membrane.

The diaphragm cells have a general resemblance to the blood cells in size and staining reaction, and in the large size of the nucleus as compared with that of the cell body. For this reason Anglas (1) has assumed that the diaphragm cells give rise to blood cells (leucocytes). This may perhaps be possible, although the diaphragm cells are as a rule slightly larger than the blood cells. Moreover, the latter show such frequent mitoses that an independent origin for them need not be assumed.

Accompanying the dorsal diaphragm and forming an integral part of it are extremely delicate muscle fibers (fig. 5, A, *DphCl*), the so-called alary muscles, apparently unicellular and corresponding histologically to the fibrillae of other muscles. These fibers or fibrils are attached at more or less regular intervals to the ventral wall of the heart, the line of attachment coinciding with that of the membranes. The fibers corresponding to each chamber of the heart converge to their intersegmental points of attachment of the diaphragm on the body wall, thus giving the fibers the well known fan-like arrangement (Pl. 7, A). The relation of the diaphragm muscles to the membranes of the diaphragm does not seem to be uniform. At some points the muscles appear to run between the two membranes, at others dorsad to them. Thus far the description of the structure of the dorsal diaphragm of the larva corresponds with the account given by Snodgrass (46) for the imago. In the latter, however, the dorsal diaphragm terminates in the 7th abdominal segment, since the segments remaining are highly modified. The histological elements are the same in both, except that this structure in the imago is much more robust than in the larva, and can easily be seen on dissection, while in the larva it is so delicate that special methods are necessary to demonstrate it. In regard to one feature, a discrepancy exists between the two accounts. Snodgrass speaks of the diaphragm cells as situated upon the membrane, whereas in both cases they undoubtedly lie between two membranes.

The dorsal diaphragm anterior to abdominal segment 4 is less well defined than in the segments posterior to this point, and does not form a continuous sheet, being entirely absent in the middle of the segments and restricted to triangular areas corresponding with the diaphragm muscles (Pl. 7, A). These

areas naturally diminish in size with the diminishing size of the trunk segments. The whole of this anterior half of the dorsal diaphragm is, however, so exceedingly delicate that it is difficult to determine accurately its structure in detail. In preparations showing the dorsal diaphragm in surface view, such as that from which Plate 7, A, was taken, in the segments anterior to the 5th abdominal segment the dorsal diaphragm appears as a cobweblike structure in which only the radiating muscle fibers can be clearly discerned. In sections the muscle fibers may occasionally be found, and here and there are indications of an exceedingly fine structureless membrane; but the most conspicuous elements of the anterior half of the dorsal diaphragm are certain cells which may be called the anterior diaphragm cells. These are of relatively large size, and of pale appearance in stained preparations. The cytoplasm, besides being more transparent or less deeply stained, offers no peculiarities, and has a rather finely granular appearance. The nuclei are elliptical and, unlike those of the posterior diaphragm cells, are rather small as compared with the cytoplasm. In form these cells are irregular, although always flattened in a dorso-ventral direction. Their distribution is apparently scattered and irregular; some are found close to the heart wall, some at a considerable distance from it. Occasionally two or more are found associated together in small groups, attached end to end, but usually they occur singly. They never occur in long bands, as do the posterior diaphragm cells. Although they are frequently found crowded in among fat cells, it is usually possible to discern a connection with either the diaphragm muscles or the diaphragm membrane, if not with the heart itself.

The homology and function of these cells is unknown. On the basis of a study of the dorsal diaphragm, the writer (36), following Carrière and Bürger (7), thought that but two kinds of cells, exclusive of muscle cells, were found in the dorsal diaphragm; small epithelial cells, forming the major portion of the diaphragm, and a smaller number of large cells, assumed to be homologous with the "paracardial" cells found by Heymons (14) in certain Orthoptera. The conditions found in older larvae, as described above, make this view untenable. Definite information regarding the function and homologies of the two kinds of diaphragm cells in the larva of the honeybee will undoubtedly demand extended investigation.

#### VENTRAL DIAPHRAGM

The ventral diaphragm is well developed in newly-hatched larvae (36) and in the imago (46) and forms a continuous sheet made up of transversely arranged muscle fibers overarching the ventral nerve cord and partitioning off a ventral (perineural) sinus. In older larvae it becomes merely a vestigial structure confined to the abdominal segments, and composed of more or less isolated delicate muscle fibers crossing the body cavity above the ventral nerve cord. They are entirely too few in number to form anything even approaching a membrane in structure, and constitute only a very loose and insignificant meshwork (Pl. 5, A, *VDph*).

#### BLOOD

The blood comprises a transparent fluid, the plasma, watery and only slightly viscid, and the blood cells (blood corpuscles, leucocytes) or amoebocytes. In the feeding larva the latter are of one sort only and correspond to the young amoebocytes of Cuénot (8). The amoebocytes of the bee larva are scattered throughout the spaces filled by the blood, but are usually more numerous in the dorsal sinus in the region of the heart. They vary in form from ovoid or ellipsoid to spherical, but the latter form is assumed only by cells preparing to divide. The ovoid form (fig. 5, C) is the one most frequently assumed by resting amoebocytes. In narrow spaces these are sometimes found slightly deformed by

pressure from the adjoining tissues, but no evidence of a true amoeboid movement was observed. Unlike the cells of many other organs and tissues of the larva, such as those of the mid-intestine and of the fat body, the size of the amoebocytes remains nearly constant throughout the life of the larva, and is from 6 to 9 microns wide and 7 to 12 microns long. The cytoplasm stains moderately with the usual chromatin stains and normally presents no evident differentiations or inclusions. The nucleus is relatively very large, is spherical in form, and contains a few small chromatin granules. Cells in mitosis are quite frequent (fig. 5, D-H) and it is easy to find a series representing the different phases. This seems to be the normal if not the only mode of increase, since there is no good evidence of a special blood-forming tissue in the bee larva.

Anglas (1) describes a second type of blood cell "lymphocyte," larger, and with the cytoplasm finely vacuolated. This type apparently does not appear in the larva prior to capping.

The action of the heart and the flow of the blood are very similar to those of the imago, as described by Snodgrass (46), except that the sole propulsive organ is the heart. Except in very young larvae, the ventral diaphragm is so poorly developed that its action must be practically negligible. In a mature living larva, removed from the cell, successive waves of contraction are seen to pass cephalad over the heart at the rate of about one per second at room temperature, driving the blood into the head cavity. From here the blood flows caudad, filtering back through the channels formed by the various spaces between the fat body and the viscera. Some of these spaces are sufficiently well defined to deserve to be called blood sinuses. The most conspicuous of these is the ventral sinus (Pl. 5, A, VS), a tubular space extending the entire length of the larva above the ventral nerve cord and bounded laterally and dorsally by lobes of the fat body. As the figure shows, it is not limited dorsally by the ventral diaphragm (VDph), as it is in other insects. Another considerable space is found between the heart and the alimentary canal, while a continuous space exists around the mid-intestine, permitting a free flow of blood over the external face of the latter. Around the tracheal trunks and their principal branches are also narrow spaces free from fat cells, which form blood channels. The pericardial chamber is more or less completely filled with fat cells (the pericardial fat cells) except in the middle of each segment, where there is a space on each side of the heart, opposite the ostia, which is unoccupied by fat cells. These spaces extend laterad from the heart a short distance, and each receives one of the dorsal tracheal branches (fig. 2, A), being thus connected with the blood channel accompanying this branch. In the living larva blood may be seen flowing dorsad along this path. The pericardial blood lacuna just described may be seen in Plate 7, A, in which the round pericardial cells are represented in dotted outline. It should be stated, however, that these cells actually extend farther laterad than is shown, many of them having been removed in dissection. In the living larvae, and even in some fixed specimens, these pericardial lacunae are evident externally as short dark bands extending right and left from the heart in each segment (see Pl. 1, E).

## FAT CELLS, OENOCYTES, EXCRETORY CELLS

### FAT CELLS

At all stages except the earliest, fat cells, constituting collectively the so-called fat body, occupy approximately a half of the body cavity not already occupied by the other viscera. In life the fat cells are nearly opaque and colorless. Since they underlie the transparent hypodermis over the greater portion of the body they are largely responsible for the ivory-white tint of the larva. In general, the fat cells adhere together in flat lobular masses or strings, supported by tracheae.

and projecting centrad into the body cavity (Pl. 5, A, 1-3*F*). In the arrangement of these masses a certain amount of bilateral symmetry exists. Carrière and Bürger (7), in their description of the larval mason bee, divide the fat body into three sections: (1) That part, situated dorsad to the lateral tracheal trunks and below the dorsal diaphragm (Pl. 5 A, 1*F*); (2) that part situated ventrad to the lateral tracheal trunks (2*F*); (3) that part situated above the dorsal diaphragm and comprising one to three layers of cells (3*F*). These divisions are only incompletely separated and are merely of topographic value, since there are no differences in the morphological character of the cells themselves. As shown in Plate 5, A, the fat cells are most abundant peripherally, next to the body wall, a considerable space around the alimentary canal being left vacant (see p. 1184). In advanced larvae fat cells are abundantly present in contact with the hypodermis, but not attached to it, throughout the trunk, crowded between the hypodermis and muscles and also beneath the ventral nerve cord. A few fat cells are likewise present in the head, lying against the posterior surface of the brain and below the suboesophageal ganglion. The various open spaces in the fat body, constituting channels for the flow of blood, have been described in a previous section (see p. 1199).

Bishop (4) has published a detailed account of the structure and development of the fat cells of the honeybee larva, to which the reader is referred for more extended information on this subject. In quite young larvae the deutoplasm of the fat cells (Pl. 8, D and E, *F*) is commonly found represented in each cell by a single relatively large vacuole (*y*), while the nucleus is still more or less spherical. In larvae three to four days old the deutoplasm of the fat cells (Pl. 8, F, *y*) is scattered throughout the cytoplasm in the form of vacuoles of various sizes; at the same time the nuclei begin to show distortion. Plate 8 illustrates a typical fat cell (*F*) from a mature larva, in which the nucleus has assumed the shrunken and branching form characteristic of this stage.

#### OENOCYTES

The oenocytes of the honeybee have been described by Wielowieysky (52), Koschevnikov (27), Anglas (1), and the writer (36). They are very conspicuous in sections, on account both of their large size and their great affinity for stains. They are rare in the thoracic segments, but are found abundantly throughout the abdomen, always occurring singly, never in groups, and generally, if not invariably, in more or less intimate contact with fat cells. In mature larvae the oenocytes closely approximate the fat cells in size, but this is not true of younger larvae. In a larva two days old, for example, the average diameter of the oenocytes is about twice that of the fat cells (Pl. 8, D, *Oen*). The rate of growth of the fat cells therefore greatly exceeds that of the oenocytes. Generally speaking, the oenocytes are more or less ellipsoid in form, but modifications of this are frequent and of such a kind as to afford plain evidence of the amoeboid, wandering nature of these cells. The oenocyte represented in Plate 8, B, is deformed to accommodate itself not only to the two adjacent fat cells (*F*, *F*) but also to a blood cell (*BIC*). It is to be noted that the contour of the fat cells is not evidently altered. Many oenocytes are found with a pointed process at one end—evidently a pseudopodium—extending between the adjacent fat cells, as though wedging them apart, while other oenocytes possess two such processes which are applied to and partially surround one or more of the adjacent fat cells. One instance of the amoeboid character of the oenocytes, as seen in sections, was particularly striking; here an oenocyte had assumed the form of a crescent embracing a fat cell between its horns, thus irresistibly suggesting the movements of an amoeba preliminary to ingesting its prey. The oenocytes, however, never



contain food vacuoles, nor do they in fact give any good evidence of being phagocytic in character. This is in accord with the observations of Anglas (1) on the wasp and honeybee and Pérez (40) on the ant.

The nuclei of the oenocytes are spherical or ellipsoid and contain a large number of subequal chromatin granules uniformly distributed (Pl. 8, B, *Oen*). The cytoplasm is dense, staining intensely in both haematoxylin and eosin, and exhibits variations in density which give it a characteristic mottled or marbled appearance.

#### EXCRETORY OR URATE CELLS

In the mature larva of the honeybee these cells, as stated by Anglas, occur only in small number. They are found in the abdomen, interpolated singly here and there among the fat cells, with which they are always closely associated. As shown in Plate 8, C, *u*, they are cells of somewhat irregular form, having a diameter about half that of the fat cells. The nucleus is spherical and contains a relatively scanty amount of chromatin in the form of small granules. The cytoplasm has a fine and regular alveolar structure, and in sections usually appears pale, having slight affinity for stains. In a very young larva (two days old) the excretory cells (Pl. 8, D, *u*) appear to be more numerous than in older larvae, and may be found in groups or clusters. They are here rendered more conspicuous both by their relatively greater size, which is equal to that of the fat cells, and more particularly by the greenish-yellow color of their cytoplasm. Under high magnification (1/12 homo. immers.) minute refractive greenish particles may be seen within the alveoles.

The general resemblance of the excretory cells to the fat cells and their close association with them at this stage argues strongly for a community of origin, as assumed by both Pérez (40) for the ant and Anglas (1) for the wasp and bee. Berlese (3) suggests that the excretory cells and the oenocytes have had a common origin. In Plate 8, D, an oenocyte from the same section as the other cells is introduced for comparison. The discrepancy in size and in other characters between this cell on the one hand and the fat and excretory cells on the other is sufficiently obvious. Moreover, the oenocytes are well differentiated and possess most of their distinctive characters even at the time of hatching (36).

#### RUDIMENTS OF GONADS

##### OVARIES

In the mature worker larva the ovarian rudiments are two small flat structures, one situated on either side of the dorsal mid-line in the 5th abdominal segment (Pl. 8, G, *Ov*). In side view each of these rudiments (Pl. 8, I) presents a trapezoidal outline, the dorsal and ventral margins being parallel, while the two ends are abruptly narrowed to form slender tapering filamentous processes which extend cephalad and caudad along the ventral side of the heart into the 4th and 6th abdominal segments respectively. Exclusive of these processes, the length of the rudiment does not exceed half of that of the segment in which it lies. The ventral margin is thickened, the dorsal margin thin, so that in section each rudiment presents a pyriform outline (Pl. 8, G and H, *Ov*).

Histologically each rudiment is composed principally of a close network of minute branching connective tissue cells (Pl. 8, H) enclosed by an epithelium made up of minute flat cells and covered externally by a cuticular tunic. From the dorsal border delicate cell-strands, clothed with a membrane continuous with the tunic, pass to the ventral wall of the heart on either side of the mid-line, thus acting as suspensory ligaments. The dorsal borders of the rudiments lie close to the mid-line, but their ventral borders lie farther apart, so that the mesial surfaces

of the rudiments are inclined at an angle of about 45 degrees to the median plane (Pl. 8, G, *Ov.*). The ventral half of each rudiment is traversed at right angles to its long axis by a large number of slender parallel columns or strings of cells with deeply stained cytoplasm, each column consisting of several rows of such cells (Pl. 8, H). These columns are assumed to be the rudiments of the ovarian tubules of the imaginal ovary. Their ventral ends extend to the ventral borders of the ovarian rudiments; the spaces between them are filled with branched connective tissue cells.

Along the ventral margin of each ovarian rudiment is a groove, at the bottom of which is located a ridge (Pl. 8, H, *Ovd*) composed of minute vacuolated cells. The base of this ridge is closely associated with the ventral or distal ends of the ovarian tubules. Near the posterior end of the rudiment the ridge becomes separated off as a solid cord of cells, taking a ventrad and caudad direction (Pl. 8, I and K, *OvD*), evidently becoming the rudiment of the oviduct.

Numerous tracheoles are found within the connective-tissue matrix surrounding the ovarian tubules. In some cases end cells have been observed within the rudiments of the ovaries.

In a mature larva, reared in a queen cell, the rudiments of the ovaries are long reniform structures (Pl. 8, L, *Ov*), measuring about 2.25 mm. in length and attached to the ventral wall of the heart in the 5th abdominal segment. The anterior ends of these rudiments, which lie on the boundary between the 4th and 5th abdominal segments, are each prolonged to form slender pointed processes like those found in the worker larva. With the exception of these insignificant processes the entire ovarian rudiment of the queen larva is composed of transverse parallel columns of dark-staining cells, assumed to represent the future ovarian tubules, similar to those of the worker larva but far more numerous, of larger diameter, and extending from the ventral to the dorsal margin. These are of course united by a connective tissue network. The (presumptive) rudiments of the oviducts are similar to those of the worker larva.

#### TESTES

These are relatively enormous structures, measuring about 3.75 mm. in length and 1.25 mm. in breadth, and lie on either side of the heart in the 4th, 5th and 6th abdominal segments, close to the dorsal body wall. They are elongate reniform in outline (Pl. 8, J, *Tes*) and somewhat compressed in a dorso-ventral direction. Like the ovarian rudiments, those of the testes are made up of numerous parallel transverse cell strands bound together by connective tissue. The (presumptive) rudiments of the vasa differentia (*VDef*) are similar to those of the oviducts in the worker and queen larvae, but more delicate and more difficult to demonstrate.

#### TECHNIQUE

Several fixing fluids were tried for killing and fixing bee larvae, but none gave as general satisfaction as Carnoy's acetic-alcohol mixtures. Their success is largely due to their superior power of penetration, since the chitinous cuticle of the larva, although very thin and delicate, is almost impenetrable by aqueous solutions.

The study of the internal organs by dissection is made difficult by the short and curved form of the larva, its small size, the abundance of fat cells and the general delicacy of the tissues. The preparation represented by Plate 4, A, was made from a larva fixed in Carnoy's fluid, stained over night in Mayer's carmalum and heavily destained (4 to 6 hours) in acidulated alcohol. The body wall, muscles, coagulated blood, etc., were then removed bit by bit with a needle, the stain rendering possible the identification of the different organs and tissues.

This method of dissection was the one usually employed. Fresh material was not found satisfactory. Very successful entire preparations of separate parts, such as the head, were made by staining for 18 to 24 hours in carmalum and afterwards destaining in acidulated alcohol (0.1 per cent) for about the same length of time. To demonstrate the dorsal diaphragm, the larva was first killed and the body wall and muscles fixed by immersion in absolute alcohol for five minutes. The dorsal body wall was then removed with the aid of fine scissors, spread out on a slide, ventral surface uppermost, and the fat body gently pushed away from either side of the dorsal mid-line, thus uncovering the dorsal diaphragm. The latter was then fixed with any convenient fixing fluid, stained rapidly (5 minutes) with Ehrlich's haematoxylin, dehydrated, cleared, and mounted, together with the dorsal body wall, to which it remains attached.

Sections were made in the usual manner. Celloidin sections 30 to 40 microns thick were found very useful in determining the relation and size of organs. Sections of material imbedded in paraffin were cut 6 to 8 microns in thickness, since the completeness of the series was usually more important than extreme thinness of individual sections. Prior to embedding, the larvae were either cut in half, or, when sagittal sections were desired, an aperture of considerable size was made in the lateral body wall. Infiltration is complete in 4 to 6 hours, if xylol has been used for clearing.

Iron haematoxylin proved to be the only satisfactory stain for sections of material embedded in paraffin, the other haematoxylin stains being too diffuse in their action. A counter stain such as Orange G or alcoholic solution of eosin is useful in differentiating certain structures, such as nerve fibers or the albuminoid granules of the fat cells. Material intended for celloidin sections was stained in bulk with Mayer's carmalum.

### SUMMARY

(1) The bee larva has a fusiform shape, the posterior end being the smaller, and is flexed ventrad. Its color is ivory white. The larva is divided by constrictions into a head and 13 segments, 3 of which belong to the thorax, the remainder to the abdomen. The sternal surface of the abdomen is sharply demarcated from the lateral surfaces by the ventrolateral suture. The ventrolateral region of abdominal segments 1 to 9 is raised to form a series of rounded swellings, the epipleural lobes.

Ten pairs of spiracles are present, situated on the lateral faces of the 1st and 2d thoracic and the first eight abdominal segments, near their anterior limits. The head is short and blunt and the neck constriction is a narrow fold. The labium is prominent, bluntly conical, slightly compressed dorso-ventrally, and bears on its tip the common opening of the silk glands. The maxillae and mandibles are papillate, the mandibles being more pointed than the maxillae and curved mesiad. A well-defined groove, the lateral furrow, runs from between the bases of the mandibles and maxillae caudad to the neck constriction. The labrum is broad and flat, slightly bilobed at its apex, and is indistinctly marked off from the clypeus. The labrum and labium are separated by a narrow cleft-like space, the mouth opening, which is bounded laterally by the mandibles and maxillae. On each side of the clypeus the antennal rudiments are evident as circular papillate elevations.

(2) The body wall consists of a single epithelial layer of small cells, the hypodermis, clothed externally by a delicate cuticle. The hypodermis differs greatly in thickness in different parts of the body but its average thickness is greatest in the head. The cuticle also is here thicker and more rigid than elsewhere. The antennal rudiments are ovoid in form and situated in deep peripodal cavities,

only the peripheral ends of the rudiments projecting above the surface of the head. The peripodal cavities of the antennae as well as of the other appendages are closed externally only by cuticle. The wing rudiments are small, flat, heart-shaped, hollow outgrowths of the hypodermis situated in shallow depressions low down on the second and third thoracic segments. The leg rudiments are ovoid in shape and are situated in deep peripodal cavities on the ventral side of the three thoracic segments, close to the mid-line. The genital rudiments are six in number, one pair being situated on the 8th abdominal segment and the other two pairs on the 9th. They are knob-like in form and lie in shallow open depressions.

(3) The rigidity of the head capsule is increased by the tentorium, which consists of a system of tubular ingrowths of the hypodermis lined by chitin. It comprises two short and broad posterior arms which are attached to the cranial wall at the junction of the lateral furrows with the neck fold; a transverse central body, continuous with the lateral arms; and two slender anterior arms joining the ends of the central body to the cranial wall at the sides of the clypeus. On the lateral face of each of the anterior arms is a spine for the attachment of a muscle. At the base of each of the mandibles, on its mesial side, is a hollow spine-like apodeme for the insertion of the adductor muscle.

(4) The nervous system is simple and primitive as compared with that of the adult and consists, in the mature larva, of a brain, a nerve chain comprising 11 ganglia joined by paired connectives and a stomatogastric ganglion with its accompanying nerves. The brain includes a pair of large somewhat crescentic or auriculate optic lobes, situated in the transverse plane, well-developed protocerebral lobes, and small antennal lobes (deutocerebrum) and tritocerebral lobes. The two halves of the latter are united by a distinct suboesophageal commissure which is not fused with the suboesophageal ganglion. Two pairs of nerves spring from the brain proper: the antennal nerves, which connect the antennal lobes with the antennal rudiments, and the labrofrontal nerves, which spring from the tritocerebral lobes. Each of these last mentioned nerves divides near its point of origin into the frontal nerve, which runs mesiad to the frontal ganglion, and the labral nerve, which innervates the labrum. The suboesophageal ganglion, attached to the brain by slender crura cerebri, is somewhat lenticular in form, broader at its anterior end. It represents three pairs of simple ganglia and gives rise on each side to four nerves: mandibular, maxillary, labial, and the X-nerve of Jonescu. The last mentioned possibly corresponds to the salivary gland nerve in other insects. In the bee larva it is difficult to trace but appears to terminate in the superficial hypodermis at the base of the labium. The 11 ganglia of the ventral cord are lenticular in form and are connected with one another by distinct parallel connectives. The ganglia are not located in the middle of the segments but are near their anterior ends. The three thoracic ganglia are the largest, those of the following seven abdominal segments being subequal; the 8th abdominal ganglion is, however, elongate and comprises three pairs of simple ganglia and the rudiment of a fourth. All of the abdominal ganglia are provided with well-developed lateral nerves which divide into branches supplying the viscera, muscles, etc. The stomatogastric system comprises a well-developed stomatogastric ganglion situated above the pharynx and connected to the brain by the frontal nerves. Anteriorly it gives off a single nerve, the superior pharyngeal nerve, supplying the superior pharyngeal muscles and the tip of the labrum. Posteriorly the stomatogastric ganglion gives off a stout nerve which passes caudad, diminishing in caliber meanwhile, along the dorsal surface of the oesophagus. It finally breaks up into small branches.

The nervous system of the larva is histologically similar to that of the imago but is of course less specialized. The central nervous system displays the usual

division into an outer and cortical zone of ganglion cells surrounding an inner central core of nerve fibers (punk-substanz). Among the ganglion cells large actively dividing neuroblasts, like those of embryos, may frequently be recognized. An outer neurilemma is always distinguishable; an inner neurilemma is probably also constantly present, but less evident. In the brain of the larva many of the typical features of the imaginal brain may be readily recognized, such as the arrangement of the cells of the optic lobes to form inner, middle and outer fibrillar masses, and the presence of well-developed mushroom bodies.

(5) The corpora allata are spherical bodies about 0.85 mm. in diameter, situated one on each side of the mid-line, close behind the brain, lying on the dorsal side of the anterior arms of the tentorium, and in close contact with the walls of the aorta. Each is composed of a compact mass of polyhedral cells. Tracheoles may be seen to enter these bodies and penetrate between their component cells.

(6) There are ten pairs of spiracles, following the rule for insect larvae, located on the 2d and 3d thoracic and the first eight abdominal segments. The spiracles of each side open by short branches into a longitudinal trunk. The two trunks are united anteriorly by a loop or commissure in the region of the neck, above the oesophagus, and posteriorly by a similar loop below the hind-intestine. The longitudinal trunks are also connected by segmentally arranged commissures which run close to the ventral body wall. Two pairs of tracheal branches from the anterior tracheal loop supply the brain, antennal rudiments and other parts of the head. Segmentally arranged branches springing from the longitudinal tracheal trunks supply the muscles, heart and viscera. The tracheae terminate in typical tracheal end cells from which arise the tracheoles. The latter never branch or anastomose and have never been observed to penetrate the cytoplasm of other cells.

(7) The alimentary canal comprises a short and relatively slender fore-intestine, a large cylindrical mid-intestine and a hind-intestine. The fore-intestine includes mouth, pharynx and oesophagus, these three divisions being somewhat ill-defined. The mouth is a wide transverse slit passing immediately into the pharynx. The latter is provided with a well-developed epipharynx which consists essentially of a fold of the dorsal wall of the pharynx, and which is provided with numerous transversely arranged muscles uniting the lateral margins of the fold and also with levator muscles which are attached at the dorsal ends to the dorsal wall of the clypeus and labrum. In addition, the pharynx itself is provided with two sets of dilator muscles and a pair of levators. The pharynx leads directly into the tubular oesophagus, which opens into the anterior end of the mid-intestine. Here the wall of the oesophagus is reflected upon itself to form a fold which projects into the anterior end of the mid-intestine, thus constituting an oesophageal valve similar to that found in many other insects. The oesophagus is provided with a muscular coat composed mainly of transverse fibers. The mid-intestine is very capacious, cylindrical in form, about one-third of the diameter of the body and extends from the prothoracic to the 9th abdominal segment. Its walls are thick and composed of large cubical cells displaying the usual striated border. A peritrophic membrane, apparently gelatinous in consistency, lines the mid-intestine. This layer appears to be secreted by those cells of the mid-intestine lying next to the oesophageal valve. The mid-intestine possesses a muscular coat composed of an inner layer of delicate longitudinal fibers and an outer layer of transverse fibers. The muscle fibers of both layers are branched and anastomose with one another. This is more conspicuously seen, however, in the case of the inner longitudinal layer. The posterior end of the mid-intestine is completely closed and its extremity is not covered by the muscular coat. The hind-intestine is a relatively slender tube, doubled on itself

and of uniform diameter except at its anterior end, which is enlarged and closed anteriorly by a thin diaphragm-like membrane which is closely applied to the extreme posterior end of the mid-intestine. The hind-intestine is clothed with a well-developed muscular coat composed of an inner layer of circular (transverse) muscle fibers and an outer layer of longitudinal fibers. Posteriorly the hind-intestine terminates in a slit-like anus. Here the hind-intestine is provided with muscle fibers which act as dilators.

(8) The Malpighian tubules are long and winding, extending cephalad to the region of the metathorax. Their tapering and pointed posterior (central) ends are blind and are inserted between the posterior extremity of the mid-intestine and the thin epithelial layer closing the anterior end of the hind-intestine. In young larvae the Malpighian tubules are slender, with a small lumen and relatively thick walls; in old larvae they attain a relatively large diameter and possess very thin walls, being obviously much distended. After the cell occupied by the larva has been sealed and communication between the mid- and hind-intestine has been established, permitting the discharge of feces, each of the Malpighian tubules acquires an opening into the mid-intestine by means of a minute canal perforating the annular remains of the epithelial layer which formerly closed the anterior end of the hind-intestine. The discharge of the fluid excreta of the Malpighian tubules thus occurs simultaneously with the discharge of solid excreta from the mid- and hind-intestines.

(9) The silk glands comprise a pair of slender tubes, thrown into numerous short convolutions and extending nearly the entire length of the larva, below the mid-intestine. Their anterior ends unite to form a thin-walled duct, lined with chitin, which opens by a slit-like aperture on an elevation situated on the tip of the labium.

(10) The trunk muscles are the same in all the abdominal segments except the 10th, and are (1) ventral longitudinal, a group occupying the sternal region of each segment and connecting its anterior and posterior margins; (2) ventral oblique, a pair of flat bands running obliquely cephalad and mesiad across the sternal region and also connecting the anterior and posterior margins of each segment; (3) lateral oblique, comprising one stout band crossing the lateral region of each segment in a cephalad and mesiad direction and connecting the two margins of the segment, and a small band crossing the lateral region in the opposite direction and having its dorsal end attached to the body wall in the region of the spiracle; (4) two small dorso-ventral bands; (5) dorsal longitudinal muscles connecting the anterior and posterior dorsal margins of the segments; (6) dorsal oblique muscles running from the posterior margin of each segment, in the dorso-lateral region, obliquely cephalad and mesiad to an oblique line of attachment on the body wall, the sutural line separating the prescutum and the scutoscuteum. Since the body wall is nowhere rigid, the arrangement of the muscles is responsible, to a large extent at least, for the external contour of the larva.

(11) The heart is a thin-walled tube, blind at its posterior end and running the entire length of the body in the mid-line close to the dorsal body wall, and continuous cephalad as the aorta, which enters the head. The structure of the larval heart closely resembles that part of the imaginal heart lying in the abdomen. It possesses a pair of valvular ostia in every segment except the 1st (possibly also the 2d) thoracic segment and the 9th and 10th abdominal segments. The heart is composed of two rows of flat cells on each side of the mid-line. The cytoplasm of these cells is differentiated to form striped muscle fibers. Externally the heart is clothed with a network of branching minute connective tissue cells. A dorsal diaphragm is present which is especially well-developed in the 4th to the 9th abdominal segments, where it is composed largely of sinuous bands of small

cells, as in the adult. Anterior to this region a few cells of medium size, of unknown significance, are found attached to the ventral border of the heart. The ventral diaphragm is well developed in young larvae, consisting of a sheet formed of transverse muscle fibers, spanning the lower portion of the body cavity. In old larvae, however, there remain only a few scattered muscle cells. The blood cells of the larva are all of one kind, of minute size and ellipsoid form. Many are found in division, indicating that this is the chief, if not the only, method of increase, since no blood-forming tissue is found.

(12) The fat body is voluminous, occupying a large part of the body cavity. Certain spaces are, however, left open for the flow of blood. The most evident of these are, (1) a tubular space extending longitudinally beneath the alimentary canal; (2) an annular space around the mid-intestine; (3) a tubular space extending longitudinally above the alimentary canal; (4) a set of transverse spaces in the dorsal region of the body, one in the middle of each segment and communicating with the ostia of the heart. Excretory (urate) cells are present in limited number, scattered among the fat cells. Oenocytes are very abundant and conspicuous because of their large size and staining reaction. They are found scattered throughout the trunk, but are most abundant in the abdomen. They are evidently amoeboid wandering cells, but no evidence of phagocytic activities was found.

(13) The rudiments of ovaries in the worker larvae are very small and situated in the 5th abdominal segment, attached to the ventral border of the heart and consisting principally of connective tissue, in which are embedded transverse strands of minute cells. These strands are presumably rudiments of ovarian tubes. Rudiments of oviducts are present as delicate solid strands of cells. The rudiments of ovaries in the queen larva are much larger than in the worker larva, showing that their development is greatly accelerated during the later larval stages. Their structure is similar to that of the worker larva except that the presumptive rudiments of ovarian tubes are both more numerous and longer. The rudiments of testes in the drone larva are relatively enormous, lying in the 4th, 5th and 6th abdominal segments, and are composed of very numerous transversely arranged strands of cells united by connective tissue. Rudiments of vasa deferentia are present.

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## EXPLANATION OF SYMBOLS USED IN PLATES AND TEXT FIGURES

*AntL*, Antennal lobe.

*AntNv*, Antennal nerve.

*Ant*, Antennal rudiment.

*Ao*, Aorta.

*ApTen*, Spur on anterior arms of tentorium.

*ATraL*, Anterior tracheal loop.

*BIC*, Blood cells.

*Br*, Brain.

*1Br*, Protocerebral lobes.

*3Br*, Tritocerebral lobes.

*Bra1*, *Bra2*, Tracheal branches to head.

*CCer*, Crura cerebri.

*Clp*, Clypeus.

*Com*, Commissure of ventral nerve cord.

*Con*, Connective of ventral nerve cord.

*ConCls*, Connective tissue cells.

*CorAll*, corpora allata.

*Ctl*, Cuticle.

*DDph*, Dorsal diaphragm.

*DilPhy*, dilator muscles of pharynx.

*DLMcl*, Dorsal longitudinal muscles.

*DOMcl*, Dorsal oblique muscles of trunk.

*dph*, Diaphragm closing anterior end of hind intestine.

*DphCl*, Diaphragm cells of heart.

*DphMcl*, Muscle fibers of dorsal diaphragm.

*EMd*, Extensor muscle of mandible.

*EMx*, Extensor muscle of maxilla.

*EpL*, Epipleural lobes.

*Ephy*, Epipharynx.

*EphyMcl*, Epipharyngeal muscles.

*1F-3F*, Fat cells.

*f*, Fatty globules of fat cells.

*fm1*, Outer fibrillar mass of optic ganglion.

*fm2*, Middle fibrillar mass of optic ganglion.

*fm3*, Inner fibrillar mass of optic ganglion.

*FtGng*, Frontal ganglion.

*FtNv*, Frontal nerve.

*1G-3G*, First second, and third pairs of genital rudiments.

*Gng*, Ganglion.

*HInt*, Hind intestine.

*Ht*, Heart.

*HtCl*, *HtCls*, Pericardial cell, pericardial cells.

*Hyp*, Hypodermis.

*Hphy*, Hypopharynx.

*ImCls*, Imaginal cells of mid intestine.

*INlm*, Inner neurilemma.

*In*, Intima.

*1L*, *2L*, *3L*, Thoracic legs (rudiments).

*Lb*, Labium.

*LbNv*, Labial nerve.

*LevEphy*, Levator muscle of epipharynx.

*LevPhy*, Levator muscle of pharynx.

*Lm*, Labrum.

*LMcl*, Longitudinal muscles of alimentary canal.

*LmNv*, Labrofrontal nerve.

*LNv*, Lateral nerve.

*LNvF*, Lateral nerve fibers.

*1LOMcl*, Major lateral oblique muscles of trunk.

*2LOMcl*, Minor lateral oblique muscles of trunk.

*Mal*, Malpighian tubules of larva.

*mal*, Malpighian tubules of imago.

*MB*, Mushroom bodies.

*MclEnt*, Muscular layer of alimentary canal.

- Md*, Mandible.  
*MdNv*, Mandibular nerve.  
*MInt*, Mid intestine.  
*Mth*, Mouth.  
*Mx*, Maxilla.  
*MxNv*, Maxillary nerve.  
*Nbl*, Neuroblasts.  
*Nlm*, Neurilemma (outer).  
*Nu*, Nucleus.  
*NvF*, Nerve fibers.  
*O*, rudiment of ocellus.  
*Oe*, Oesophagus.  
*OeCom*, Circumoesophageal commissure.  
*Oen*, Oenocyte.  
*OeVlv*, Oesophageal (proventricular) valve.  
*OpL*, Optic lobe.  
*OpPl*, Optic plate.  
*Ost*, Ostia of heart.  
*Ov*, Ovary.  
*OvD*, Oviduct.  
*Pmb*, Peritrophic membrane.  
*PrF*, Postretinal fibers.  
*Prs*, Prescutal area.  
*PTraL*, Posterior tracheal loop.  
*qq*, Food matter in mid intestine.  
*RAp*, Retractor apodeme of mandible.  
*1RLb*, Major retractor muscle of labium.  
*2RLb*, Minor retractor muscle of labium.  
*RMd*, Retractor (adductor) muscle of mandible.  
*RMx*, Retractor muscle of maxilla.  
*Scs*, Scuto-scutellar area.  
*SDilPhy*, Superior dilator muscles of pharynx.  
*SlkD*, Common duct of silk glands.  
*SlkDO*, External opening of common duct of silk glands.  
*SlkGL*, Silk glands.  
*SoeCom*, Suboesophageal commissure.  
*SoeGng*, Suboesophageal ganglion.  
*Sp*, Spiracle.  
*SpBr*, Tracheal branch to spiracle.  
*SPhyNv*, Superior pharyngeal nerve.  
*st*, Stalk, or projection of fundus of midintestine.  
*StgNv*, Stomatogastric nerve.  
*StrBor*, Striated border of the epithelium of the midintestine.  
*SupCom*, Supraoesophageal commissure.  
*Ten*, Central body of tentorium.  
*1Ten*, *2Ten*, Anterior and posterior arms of tentorium.  
*Tes*, Testis.  
*TMcl*, Transverse muscles of alimentary canal.  
*Tra*, Trachea.  
*tra*, Tracheoles.  
*TraCom*, Tracheal commissure.  
*TraEcl*, Tracheal end cell.  
*TraTr*, Tracheal trunk.  
*U*, Urate or excretory cell.  
*VDef*, Vas deferens.  
*VDph*, Ventral diaphragm.  
*VLMcl*, Ventral longitudinal muscles.  
*VLS*, Ventrolateral suture.  
*VNC*, Ventral nerve cord.  
*VOMcl*, Ventral oblique muscles of trunk.  
*VS*, Ventral sinus.  
*WngR*, Rudiments of wings.  
*X*, Nerve designated as "X" by Jonescu.  
*y*, Food reserve in fat cells.

## PLATE 1

### Larva of the honeybee

A.—Head of mature larva, treated with caustic potash, showing endoskeleton, viewed from dorsal surface.  $\times 26$ .

B.—Brain and suboesophageal ganglion of mature larva, face view. Drawn from a wax model reconstructed from sections and verified by dissections.  $\times 40$ .

C.—Same as B, side view.  $\times 40$ .

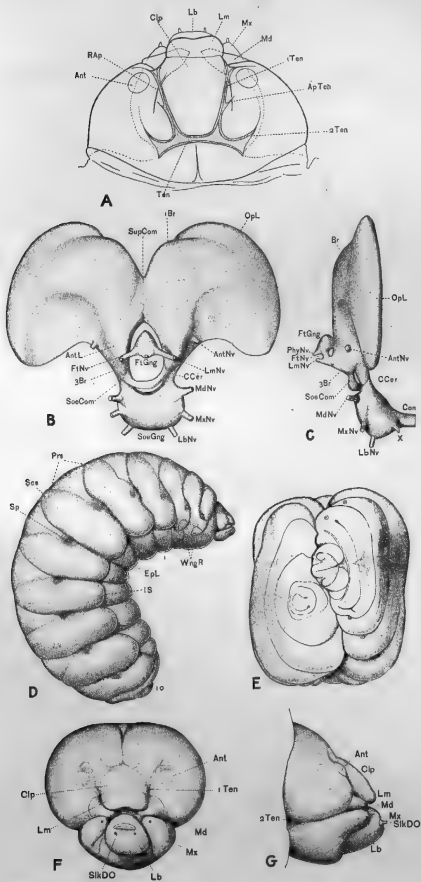
D.—Mature worker larva, removed from cell, viewed from the right side.  $\times 6$ .

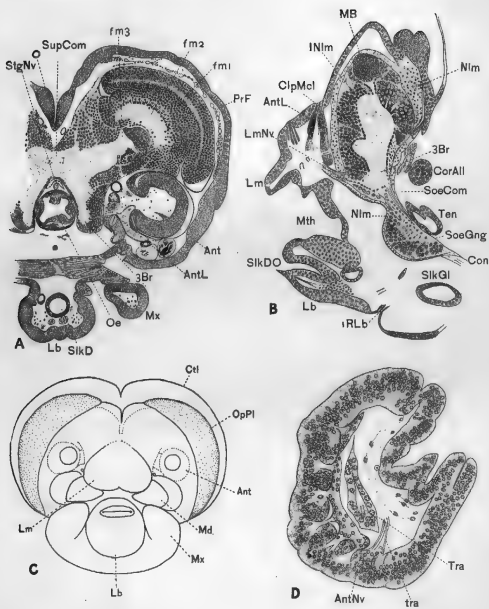
E.—Mature worker larva, fixed within the cell and afterwards removed, showing the compressed hexagonal form assumed while within the cell. The head and anus are both turned toward the observer, the body being bent in the shape of the letter U.  $\times 6$ .

F.—Face view of head of mature larva.  $\times 20$ .

G.—Head of mature larva, side view.  $\times 20$ .

(1214)





## PLATE 2

### Larva of the honeybee

A.—Cross section of head of mature larva, intersecting the optic lobes (*fm 1-3*) and antennal lobes (*AntL*).  $\times 60$ .

B.—Longitudinal section of head of mature larva, passing through one of the suboesophageal commissures (*SoeCom*).  $\times 60$ .

C.—Head of larva, face view, showing the optic plates (*OpPl*) and rudiments of antennæ (*AntR*).  $\times 40$ .

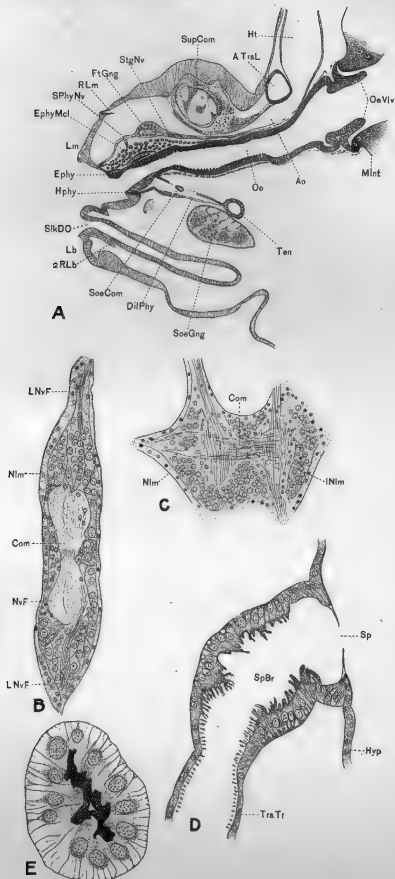
D.—Longitudinal section of one of the antennal rudiments.  $\times 156$ .

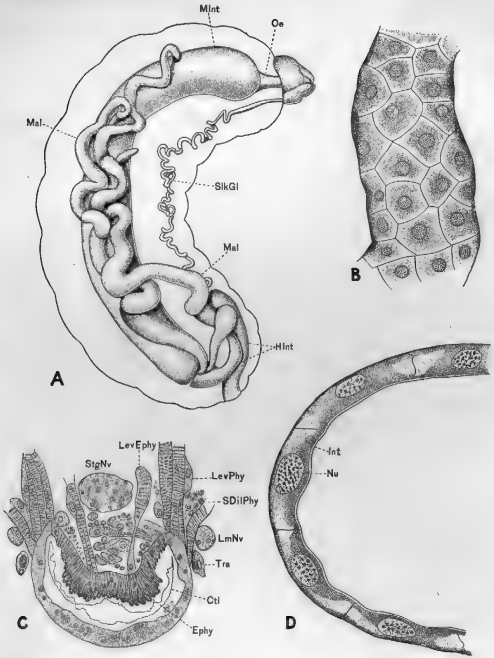


### PLATE 3

#### Larva of the honeybee

- A.—Sagittal section through head of mature larva. × 57.
- B.—Cross section of a thoracic ganglion. × 205.
- C.—Horizontal (coronal) section of a thoracic ganglion. × 153.
- D.—Longitudinal section of a spiracular branch, intersecting spiracle (*Sp*).  
× 195.
- E.—Cross section of a spiracular branch of a mature larva. × 195.





## PLATE 4

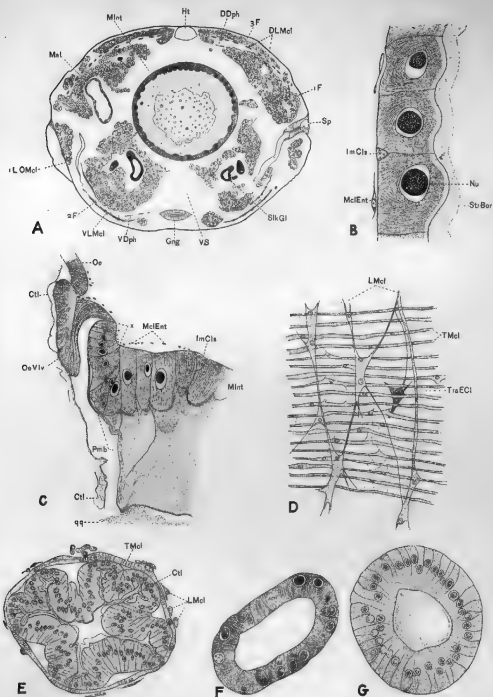
### Larva of the honeybee

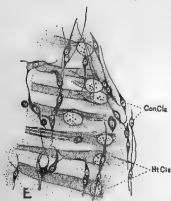
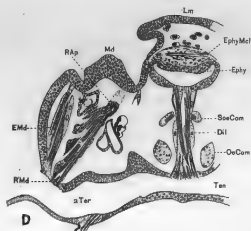
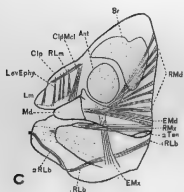
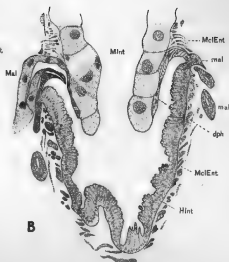
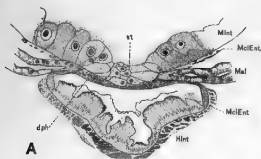
- A.—Alimentary canal, silk glands (*SlkGl*), and Malpighian tubules (*Mal*) of a mature larva, drawn from a dissection.  $\times 10$ .
- B.—Surface view of a short section of a Malpighian tubule.  $\times 75$ .
- C.—Cross section of pharynx, just caudad of frontal ganglion.  $\times 152$ .
- D.—Cross section of Malpighian tubule.  $\times 300$ .

## PLATE 5

### Larva of the honeybee

- A.—Cross section through the first abdominal segment of mature larva.  $\times 20$ .
- B.—Section of wall of mid-intestine of old larva, showing imaginal cells (*ImCls*) and striated border (*StrBr*).  $\times 235$ .
- C.—Junction of wall of mid-intestine and oesophagus, longitudinal section, showing one-half of oesophageal valve (*OeVlv*).  $\times 120$ .
- D.—Muscles of mid-intestine, seen from their inner (central) surface.  $\times ca\ 80$ .
- E.—Cross section of hind intestine, showing folds.  $\times 144$ .
- F.—Cross section of silk gland, near the fifth abdominal segment.  $\times 235$ .
- G.—Cross section of silk gland, near its anterior end.  $\times 235$ .





## PLATE 6

### Larva of the honeybee

A.—Longitudinal section through the junction of mid-intestine and hind intestine of mature larva just prior to capping of the cell. The section passes through the central blind ends of two Malpighian tubules (*Mal*), one on each side.  $\times 92$ .

B.—Longitudinal section through the junction of mid-intestine and hind intestine of a larva 12 hours after capping, showing the establishment of communication between the mid-intestine, hind intestine, and Malpighian tubules. The opening of one of the latter into the hind intestine is to be seen on the left side.  $\times 92$ .

C.—Head of larva, left side, showing musculature. From a stained and cleared preparation.  $\times 26$ .

D.—Coronal section of head of mature larva, intersecting the posterior arms of tentorium (*2Ten*).  $\times 60$ .

E.—Tangential section of wall of heart, showing cells of wall (*HtCls*) and connective tissue (*ConCls*). From the region of the fourth abdominal segment.  $\times 300$ .

F.—Tangential section of anterior end of heart.  $\times 300$ .

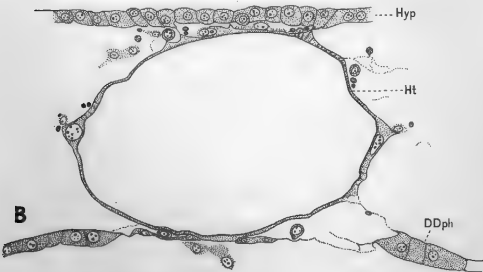
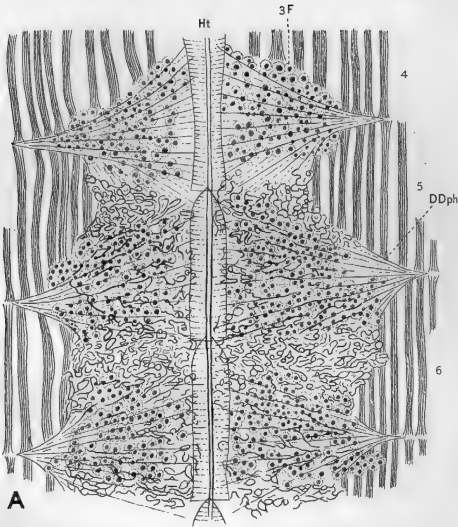


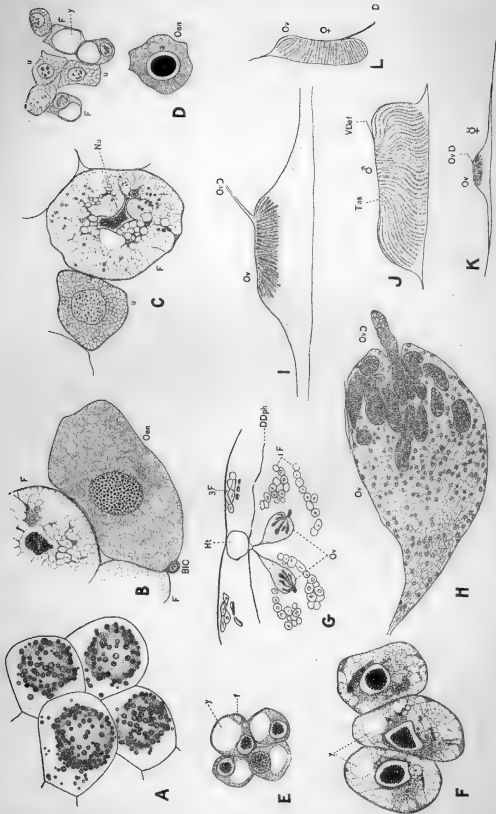
## PLATE 7

### Larva of the honeybee

A.—Dorsal diaphragm and heart, fourth, fifth, and sixth abdominal segments, viewed from the inner surface. Diaphragm cells represented by heavy wavy lines.  $\times 63$ .

B.—Transverse section of heart (*Ht*), taken near the anterior end of the abdomen.  $\times 315$ .





## PLATE 8

### Larva of the honeybee

A.—Four fat cells from advanced worker larva, showing fat globules blackened by osmic acid.

B.—Oenocyte (*Oen*) and fat cell (*F*), from a mature larva.  $\times 406$ .

C.—Urate cells (*u*) and fat cell (*F*), from a mature larva.  $\times 406$ .

D.—Fat cells (*F*), oenocytes (*Oen*), and urate cells (*u*), from a larva about two days old, showing the crowding of the nuclei toward the side of the cells.  $\times 406$ .

E.—Four fat cells from a larva 3 days old.  $\times 406$ .

F.—Three fat cells from a larva 3 to 4 days old.  $\times 406$ .

G.—Transverse section through heart (*Ht*) and rudiments of ovaries (*Ov*) of a mature worker larva.  $\times 18$ .

H.—Transverse section through the rudiment of an ovary of a mature worker larva, taken near the posterior end of the rudiment.  $\times 176$ .

I.—Side view of rudiment of ovary of mature worker larva, showing rudiment of oviduct (*Ovd*).  $\times 28$ .

J.—Rudiment of testis of drone larva, side view.  $\times 9$ .

K, L.—Rudiments of ovary of worker larva and queen larva, respectively.  $\times 9$ .

# EFFECT OF WINTER RATIONS ON SUBSEQUENT PASTURE GAINS OF STEERS<sup>1</sup>

By E. W. SHEETS

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## OBJECT AND PLAN OF THE WORK

To determine the influence of several winter rations on the gains of 2-year-old steers in the following summer on pasture, an experiment was carried on for three years. This experiment is part of a series of experiments on beef-production problems in the Appalachian Mountain region that have been in progress since December 22, 1914, in cooperation between the Bureau of Animal Industry of the United States Department of Agriculture and the West Virginia Agricultural Experiment Station, on the farm of David Tuckwiller, in Greenbrier County, W. Va. This farm is situated in the southeastern part of the State in the blue-grass area.

The results of similar work with cows, calves, and yearlings are published in United States Department of Agriculture Bulletins 1024, 1042, and 870, respectively.

The work was carried on for three years in order to have an average of feeds, cattle, seasons, and other conditions tending to produce variation. A general outline of the experiment, including the feeds used for the different lots of steers, is given in Table I.



FIG. 1.—Region to which this work applies. The black dot indicates the location of the farm on which the experiment was conducted. The shaded portion represents the area to which the results are applicable, and the dotted portion shows an additional area to which the results apply in part

<sup>1</sup> Received for publication May 19, 1924—issued Nov., 1924. This experiment is reported in full in United States Department of Agriculture Bulletin 1251.

<sup>2</sup> Arthur T. Semple and R. H. Tuckwiller, Animal Husbandry Division, United States Department of Agriculture and E. A. Livesay of the West Virginia Agricultural Experiment Station assisted in this study.

TABLE I.—Outline of the three years' work

Lot No.	Season	Steers in lot <sup>a</sup>	Winter periods <sup>b</sup>		Summer periods on pasture <sup>c</sup>
			Duration	Feed	
			<i>Days</i>		<i>Days</i>
1	1919-20	10	127	Mixed hay and ear corn.....	136
	1920-21	10	121		128
	1921-22	10	124		141
2	1919-20	9	127	Corn silage.....	136
	1920-21	10	121		128
	1921-22	10	124		141
3	1919-20	10	127	do.....	136
	1920-21	10	121		128
	1921-22	10	124		141
4	1919-20	10	127	Corn silage and cottonseed meal.....	136
	1920-21	10	121		128
	1921-22	10	124		141
5	1919-20	10	127	Corn silage, cottonseed meal, and wheat straw.....	136
	1920-21	8	121		128
	1921-22	10	124		141
6	1919-20	10	127	Corn silage and mixed hay.....	136
	1920-21	10	121		128
	1921-22	10	124		141

<sup>a</sup> Owing to accidents 1 steer died in Lot 2 in the summer of 1919-20 and 2 steers died in Lot 5 in the winter of 1920-21; averages for Lot 2 are based on 29 steers and for Lot 5 on 28 steers in subsequent tables.

<sup>b</sup> 1919-20, Dec. 23 to Apr. 27, inclusive; 1920-21, Dec. 28 to Apr. 27, inclusive; 1921-22, Dec. 23 to Apr. 25, inclusive. The average length of period is 124 days.

<sup>c</sup> 1919-20, Apr. 28 to Sept. 10, inclusive; 1920-21, Apr. 28 to Sept. 2, inclusive; 1921-22, Apr. 26 to Sept. 13, inclusive. The average length of period is 135 days.

## KINDS OF STEERS USED

The steers used were long 2-year-old grade Shorthorn, Hereford, and Aberdeen Angus, raised in southern West Virginia. They were good feeder steers, quite uniform in quality and condition. They ranged in weight from 800 to 1,200 pounds and averaged 964 pounds at the beginning of the winter periods.

## METHOD OF FEEDING AND HANDLING THE STEERS

In the fall before starting the steers on winter feed they were divided into lots of 10 each. In making this division, care was taken to have the lots as nearly uniform as possible in quality, breeding, size, and condition. The different lots were given the same amount of space in an open barn and were kept under cover all winter. Water was supplied in the stalls at all times and salt was constantly available. The steers were fed twice a day.

The feed, both concentrates and roughage, was carefully weighed at each feeding. All the feeds used were of good quality. There was practically no waste. The steers were weighed for three consecutive days at the beginning and at the end of the winter period. They were also weighed once every 28 days. All weighings were made in the morning after feeding. Neck straps and disks or ear tags with numbers on them were used, so that ready identification of each individual could be made and records accurately kept.

In the spring of each year, as soon as the grass was good enough, which was usually about April 25, the steers from all the lots were turned into a rather hilly pasture of about 300 acres with no additional feed. A good growth of blue grass with white clover is found on all parts of the pasture. A small stream, which flows through the pasture, provides an abundance of fresh water throughout the summer. The soil is of limestone formation. Under normal climatic conditions there is sufficient rainfall to keep the grass growing throughout the season.

Weights were taken once every 28 days during the first summer. Afterwards the practice was discontinued on account of the loss incident to driving such heavy steers the long distance from the pastures to the scales. During the second summer they were weighed only on the sixty-second day and during the third summer only on the forty-fourth day. Three consecutive daily weights were not taken at the end of the grazing season, on account of the incident loss in weight. Each year the steers were marketed in Jersey City at the end of the summer grazing period.

#### QUANTITY OF FEED CONSUMED

Table II shows the total quantities of different feeds eaten by the various lots and the average daily ration per steer in each lot during each of the three winters. All lots except Lot No. 2 were fed to make a small gain in weight. Lot No. 1 failed to gain in weight in the winter of 1921-22 on account of the poor quality of the mixed hay. They would not eat enough of it to maintain their weight. It was planned to feed Lot No. 2 so that it would lose slightly in weight. This object was not attained the first year. The quantities of feed consumed daily were practically the same throughout the winter periods. Lot No. 3 was given practically as much silage as the steers would clean up.

TABLE II.—Average total and daily rations per steer during winters

Winter feed	Total feed per steer			Average	Daily feed per steer			Average
	1919-20	1920-21	1921-22		1919-20	1920-21	1921-22	
Lot 1:	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>
Mixed hay .....	2,540	2,403	1,860	2,268	20.0	19.9	15.0	18.3
Ear corn .....	254	242	245	247	2.0	2.0	2.0	2.0
Lot 2: Corn silage .....	3,810	3,490	3,487	3,588	30.0	28.8	28.1	28.9
Lot 3: Corn silage .....	5,080	4,840	4,478	4,799	40.0	40.0	36.1	38.7
Lot 4:								
Corn silage .....	3,810	3,490	3,683	3,661	30.0	28.8	29.7	29.5
Cottonseed meal .....	190	181	184	185	1.5	1.5	1.5	1.5
Lot 5:								
Corn silage .....	3,125	3,025	3,069	3,076	25.0	25.0	24.7	24.8
Wheat straw .....	734	698	736	725	5.8	5.8	5.9	5.8
Cottonseed meal .....	127	121	123	124	1.0	1.0	1.0	1.0
Lot 6:								
Corn silage .....	3,125	3,025	3,069	3,073	25.0	25.0	24.7	24.8
Mixed hay .....	1,016	968	749	911	8.0	8.0	6.0	7.3

As Table III shows, there was considerable variation in the nutritive ratio of the rations as fed as well as in the quantities of dry matter and digestible nutrients in them. The rations containing hay and straw were especially high in dry matter. The rations of silage alone were lowest in digestible protein, while that containing 1.5 pounds of cottonseed meal was considerably the highest. The rations containing cottonseed meal had nutritive ratios considerably narrower than the others.

TABLE III.—Quantities of dry matter, digestible nutrients, and nutritive ratios of the rations

Lot No.	Winter ration per steer	Digestible nutrients			Nutritive ratio	Feed per 1,000 pounds live weight <sup>b</sup>
		Dry matter	Protein	Carbo-hydrate equivalent <sup>a</sup>		
		<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>		
1	Mixed hay.....(18.3 pounds).....	17.0	0.63	9.08		19.0
	Ear corn.....(2.0 pounds).....	1.8	0.07	1.38		2.1
	Total.....	18.8	0.70	10.46	1 : 14.9	
2	Corn silage.....(28.9 pounds).....	7.8	0.37	4.99	1 : 13.5	30.1
3	Corn silage.....(38.7 pounds).....	10.5	0.49	6.67	1 : 13.5	40.1
4	Corn silage.....(29.5 pounds).....	8.0	0.38	5.09		30.6
	Cottonseed meal.....(1.5 pounds).....	1.4	0.53	0.61		1.6
	Total.....	9.4	0.91	5.70	1 : 6.3	
5	Corn silage.....(24.8 pounds).....	6.7	0.32	4.28		25.7
	Wheat straw.....(5.8 pounds).....	5.4	0.02	2.26		6.0
	Cottonseed meal.....(1.0 pound).....	0.9	0.35	0.41		1.0
	Total.....	13.0	0.69	6.95	1 : 10.1	
6	Corn silage.....(24.8 pounds).....	6.7	0.32	4.28		25.7
	Mixed hay.....(7.3 pounds).....	6.8	0.25	3.62		7.6
	Total.....	13.5	0.57	7.90	1 : 13.9	

<sup>a</sup> The carbohydrate equivalent is the sum of the digestible carbohydrates plus 2.25 times the digestible fat.

<sup>b</sup> This is based on the initial weights of the steers.

#### GAINS DURING WINTER AND SUMMER

The initial spring and final weights and the gains and losses in weight during each of the three years are shown in Table IV.

TABLE IV.—Average total <sup>a</sup> and daily gains during winter and summer

Lot No.	Winter feed	Season	Initial weight per steer	Weight per steer at end of winter	Winter gain or loss per steer		Weight per steer at end of summer	Summer gain per steer		Winter and summer gain per steer	
					Total	Daily		Total	Daily	Total	Daily
			<i>Lbs.</i>	<i>Lbs.</i>	<i>Lbs.</i>	<i>Lbs.</i>	<i>Lbs.</i>	<i>Lbs.</i>	<i>Lbs.</i>	<i>Lbs.</i>	<i>Lbs.</i>
1	Mixed hay and ear corn.....	1919-20	927	962	35	0.28	1,269	307	2.26	342	1.30
		1920-21	981	1,036	55	0.45	1,321	285	2.23	340	1.37
		1921-22	980	943	-37	-0.30	1,287	344	2.44	307	1.16
	Average.....		962	980	18	0.14	1,292	312	2.31	330	1.27
2	Corn silage.....	1919-20	913	950	37	0.29	1,236	286	2.10	323	1.23
		1920-21	981	927	-54	-0.45	1,290	363	2.84	309	1.24
		1921-22	983	977	-6	-0.05	1,361	384	2.72	378	1.43
	Average.....		961	952	-9	-0.07	1,298	346	2.56	337	1.30
3	Corn silage.....	1919-20	928	1,007	79	0.62	1,246	239	1.76	318	1.21
		1920-21	987	1,033	46	0.38	1,338	305	2.38	351	1.41
		1921-22	981	1,053	72	0.58	1,359	306	2.17	378	1.43
	Average.....		965	1,031	66	0.53	1,314	283	2.10	349	1.35
4	Corn silage and cottonseed meal.....	1919-20	927	994	67	0.53	1,291	297	2.18	364	1.38
		1920-21	983	998	15	0.12	1,314	316	2.47	331	1.33
		1921-22	982	1,051	69	0.56	1,373	322	2.28	391	1.48
	Average.....		964	1,014	50	0.40	1,326	312	2.31	362	1.40
5	Corn silage, wheat straw, and cottonseed meal.....	1919-20	927	1,020	93	0.73	1,270	250	1.84	343	1.30
		1920-21	987	1,049	62	0.51	1,294	245	1.91	307	1.23
		1921-22	988	1,085	97	0.78	1,365	280	1.99	377	1.42
	Average.....		966	1,052	86	0.69	1,310	258	1.91	344	1.33
6	Corn silage and mixed hay.....	1919-20	928	991	63	0.50	1,264	273	2.01	336	1.28
		1920-21	984	1,052	68	0.56	1,295	243	1.90	311	1.25
		1921-22	982	1,028	46	0.37	1,344	316	2.24	362	1.37
	Average.....		965	1,024	59	0.48	1,301	277	2.05	336	1.30

<sup>a</sup> On account of dropping fractions in the average weights of the steers as given in this table, one can not always add the weights or gains for the 3 years, divide by 3, and obtain the average given. In no case is there more than 1 pound of difference.



### DIAGRAMS OF GAINS AND LOSSES

The seven charts, Figures 2, 3, 4, 5, 6, 7, and 8 show the gains and losses of the steers by 28-day periods, except as noted under "Method of feeding and handling the steers." The first six show the effect of the six rations under comparison for the three years they were used, one chart being used for each ration. The seventh chart shows the average gains for three years for each of the six rations.

Horizontal distance on the charts indicates the number of days that the steers were fed during the three winters and pastured during the three summers. The average data on which each monthly period began are given also. The average length of the total period for the three years was 259 days, of which 124 days were in the winter period, and the remaining 135 in the summer period.

Vertical distance on the chart represents changes in the live weights of the steers. The weights corresponding to each of the horizontal lines are given along the left side of the chart. As the average initial weight of each lot varied from year to year, the average initial weight per steer for three years is used as a basis in each chart for showing the gains made by each lot each year.

Much of the difference caused by the winter feeding was overcome during the summer. On April 28 there was a difference of 95 pounds between the highest and lowest lots; on June 23, 50 pounds; and on September 10, 33 pounds.

### CORRELATIONS

On account of the variations in the average gains of the six lots during the three years, as shown in Table IV, the correlations between the winter, summer, midsummer, and total gains of all the steers based on the losses and gains of each steer have been calculated to substantiate the conclusions indicated by the average gains of each lot for three years. Consequently Figures 9, 10, 11 and 12, are presented to show the positions of the individual steers, when they are plotted according to their winter, summer, midsummer, and total variations in weight. Accordingly, the following results have been obtained:

	Winter gain	Summer gain	Total gain
Mean.....	Pounds 44.0	Pounds 297.7	Pounds <sup>a</sup> 342.2
Standard deviation.....	60.1	62.7	57.6

<sup>a</sup> The sum of the mean winter and summer gain is 341.7 while the mean for the total gain is 342.2, the discrepancy being due in all probability to the grouping of the gains in classes of 10 pounds range.

### CORRELATIONS

Winter-summer.....	-0.572	±0.034
Winter-midsummer.....	- .480	± .038
Winter-total.....	+ .429	± .041
Summer-total.....	+ .493	± .038

### REGRESSIONS

Total/winter.....	+0.407
Summer/winter.....	- .593
Midsummer total/winter.....	+ .616
Midsummer/winter.....	- .384

According to the regressions, 100 pounds advantage in weight at the end of the winter is reduced to 61.6 pounds after 54 days on grass and to 40.7 pounds after 136 days on grass. In other words, during the first 54 days on grass, 38.4 pounds is lost of 100 pounds advantage as compared with 21.3 pounds lost during the rest of the summer period of 82 days, making a total loss of 59.3 pounds for the whole summer period.

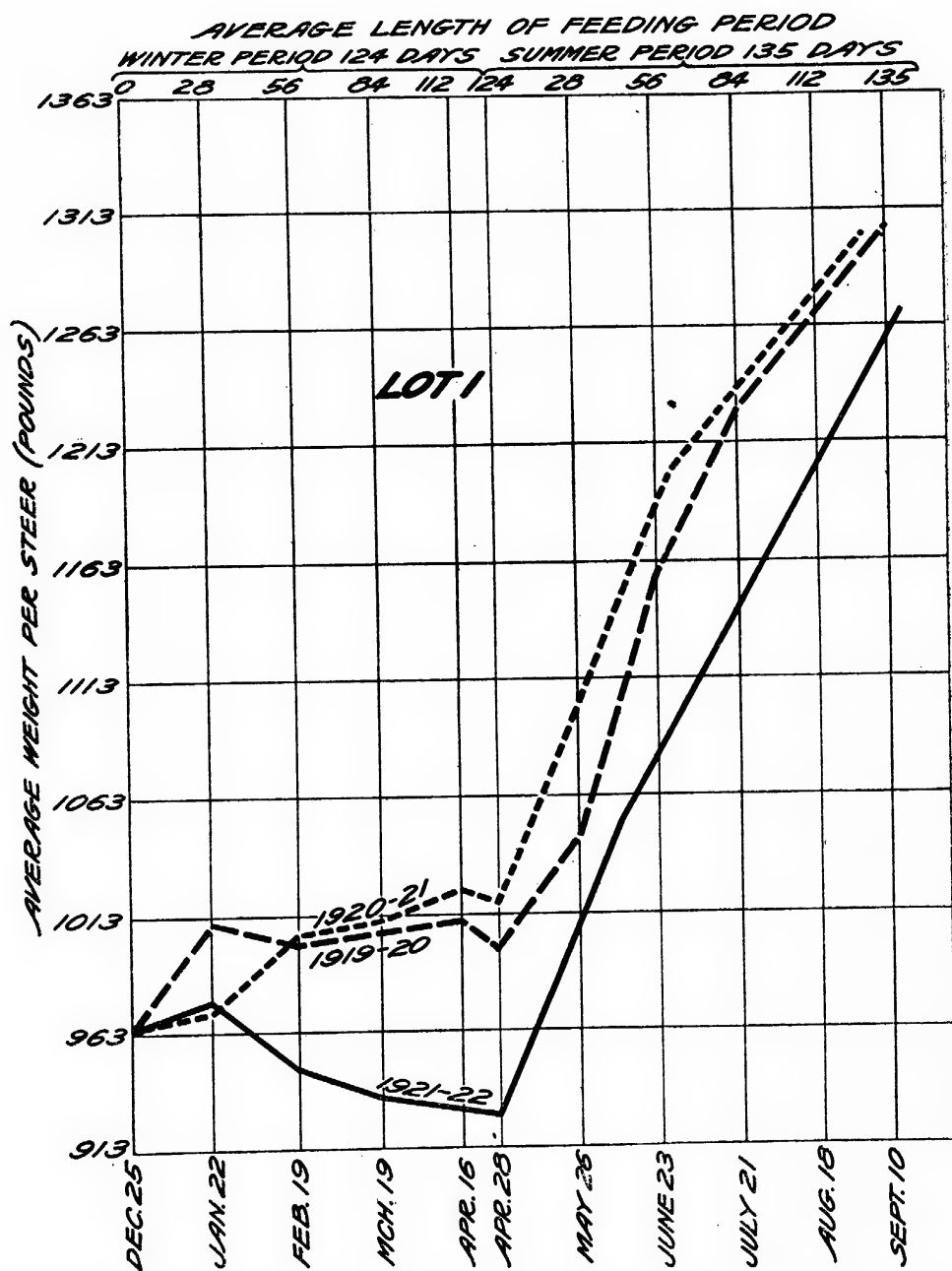


FIG. 2.—Results of winter and summer (grass) feeding for Lot 1. These steers were fed the following average ration during the winter: Mixed hay, 18.3 pounds; ear corn, 2 pounds

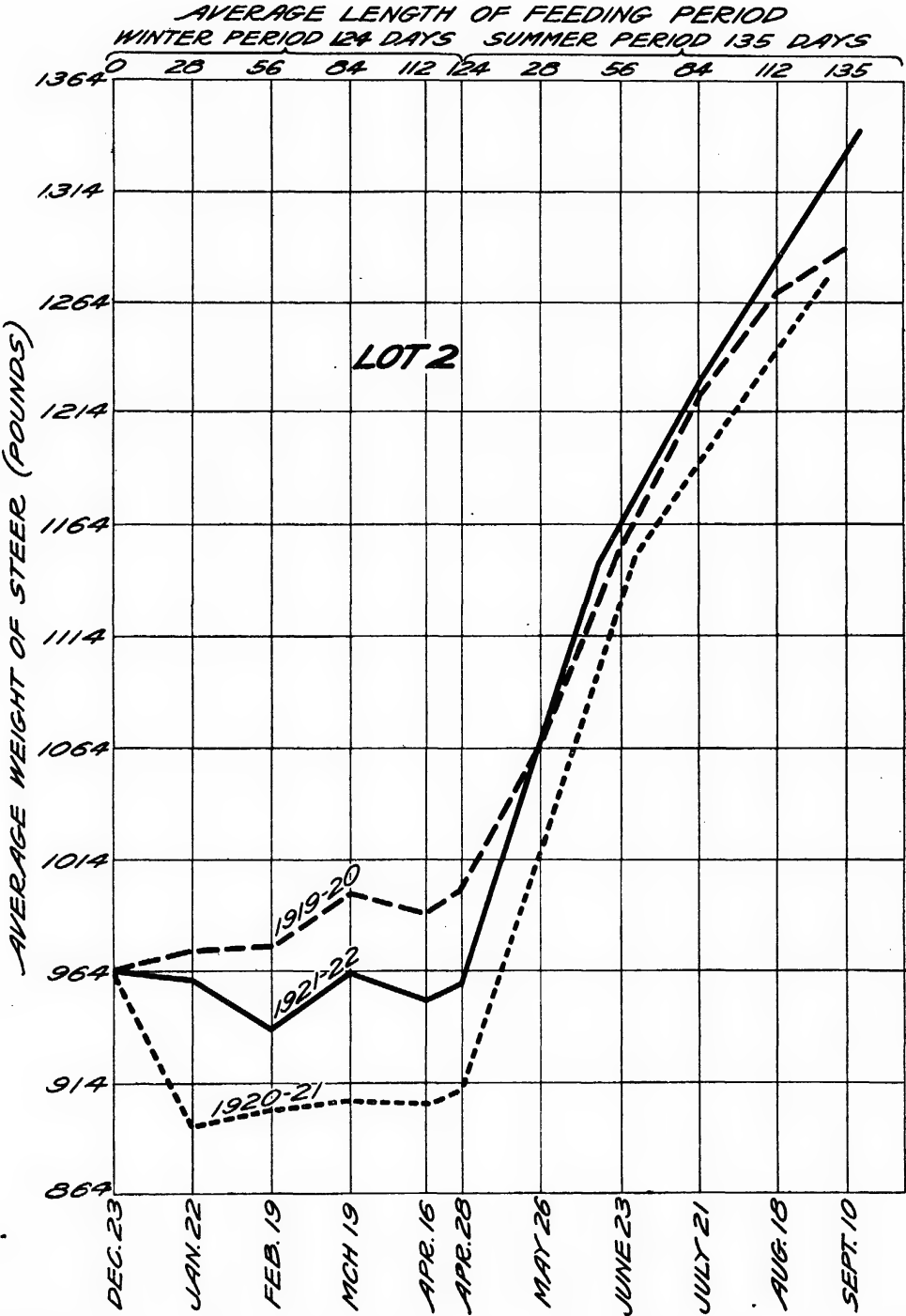


FIG. 3.—Results of winter and summer (grass) feeding for Lot 2. These steers were fed the following average ration during the winter: Corn silage, 28.9 pounds

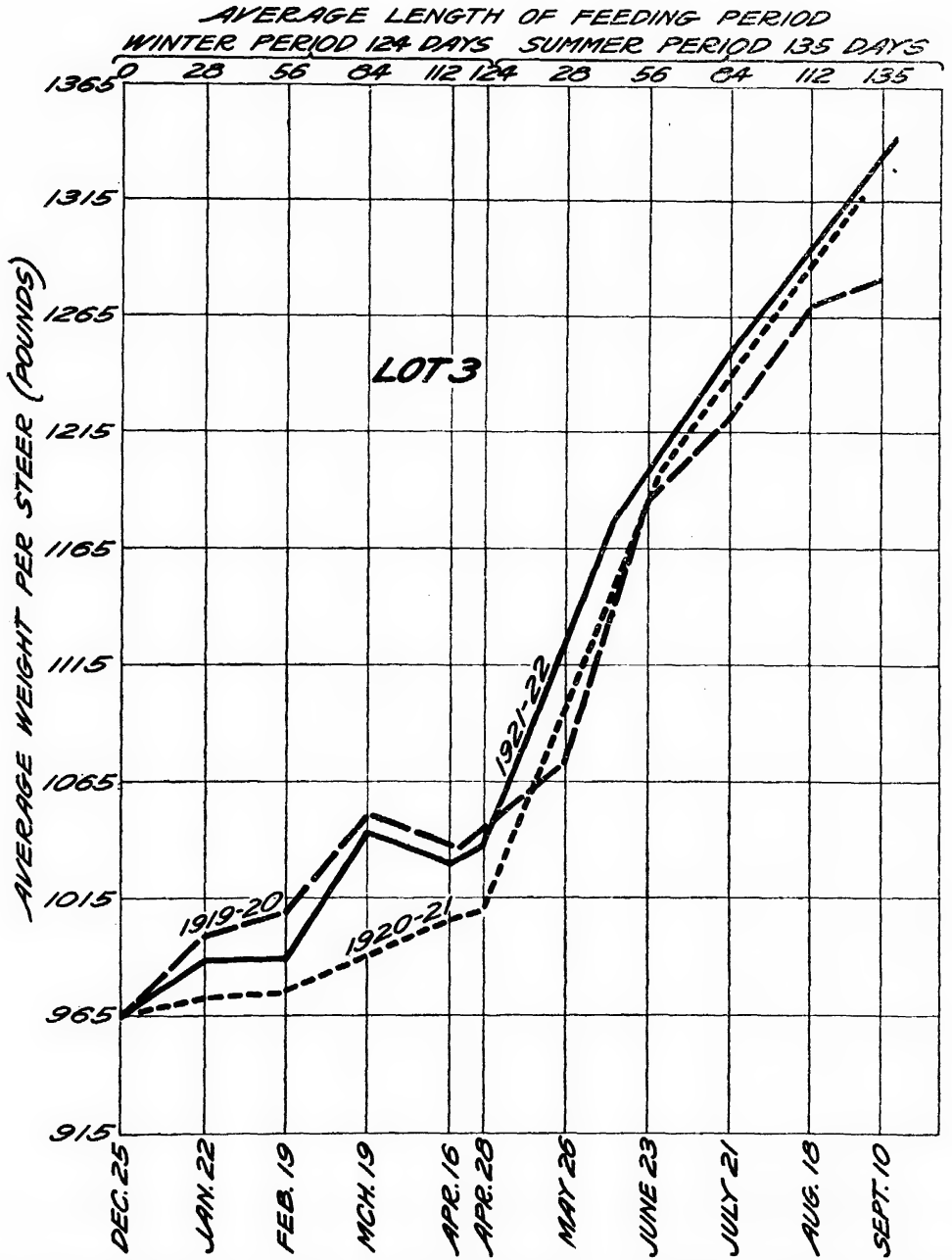


FIG. 4.—Results of winter and summer (grass) feeding for Lot 3. These steers were fed the following average ration during the winter: Corn silage, 38.7 pounds

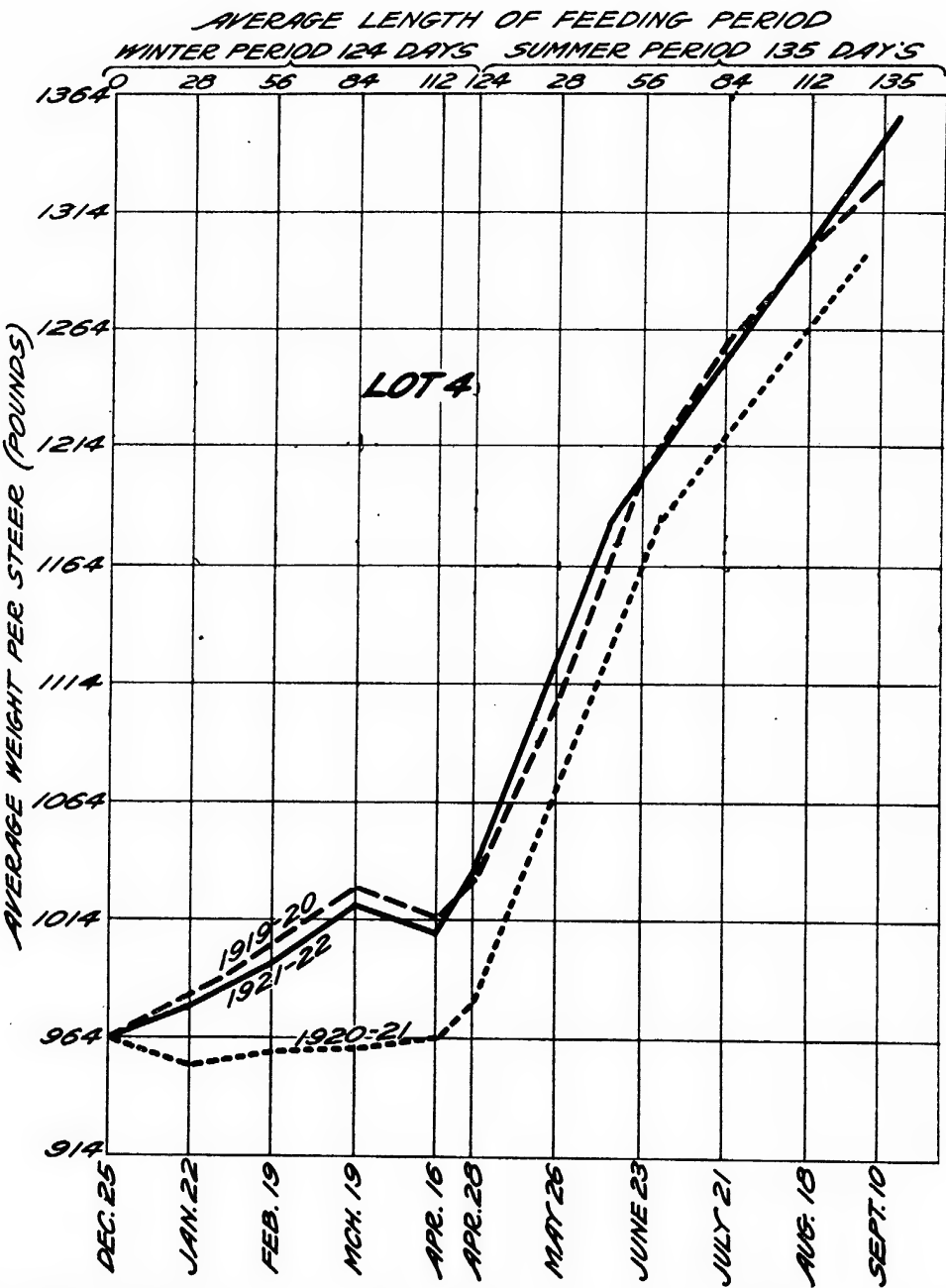


FIG. 5.—Results of winter and summer (grass) feeding for Lot 4. These steers were fed the following averageration during the winter: Corn silage, 29.5 pounds; cottonseed meal, 1.5 pounds  
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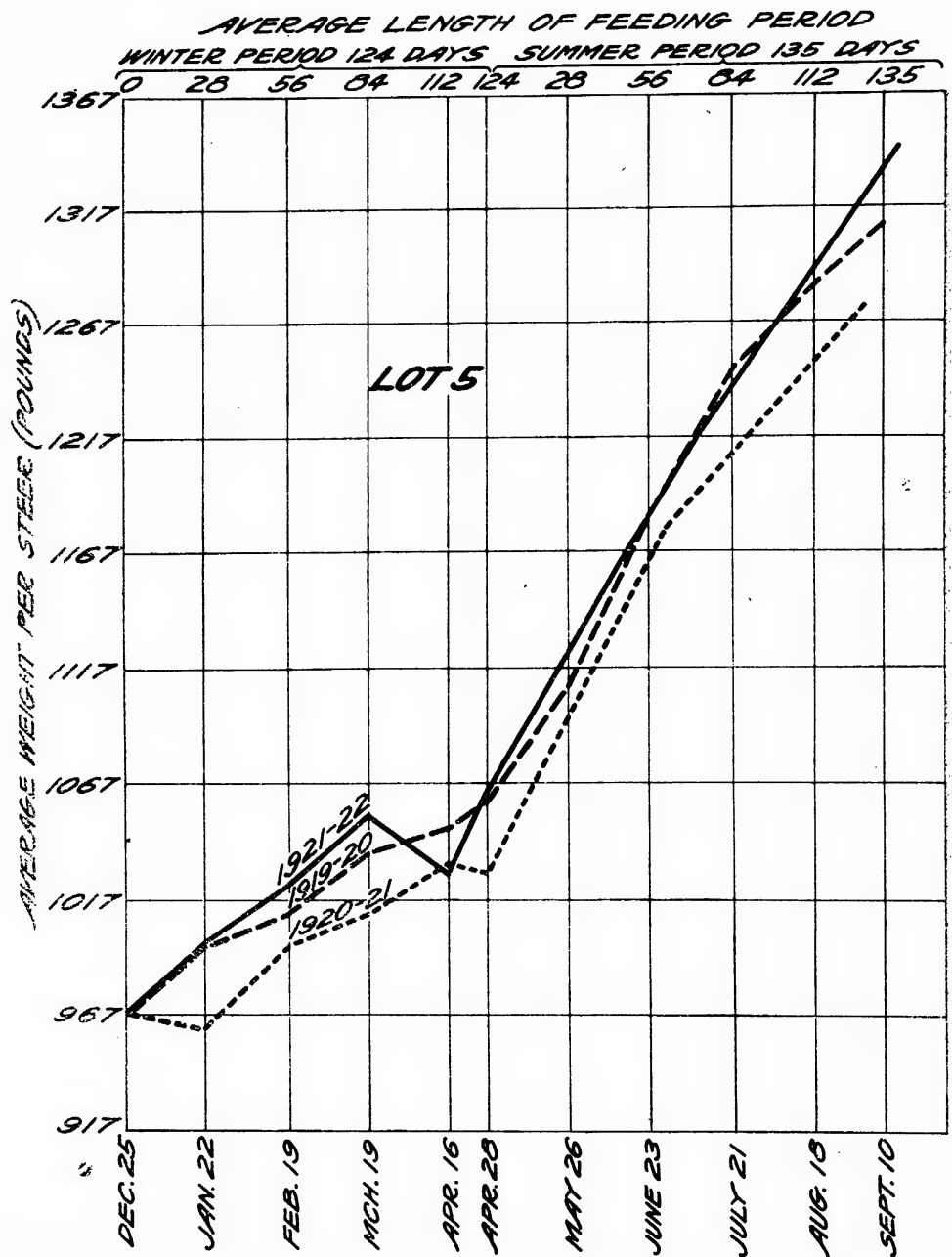


FIG. 6.—Results of winter and summer (grass) feeding for Lot 5. These steers were fed the following average ration during the winter: Corn silage, 24.8 pounds; wheat straw, 5.8 pounds; cottonseed meal, 1 pound

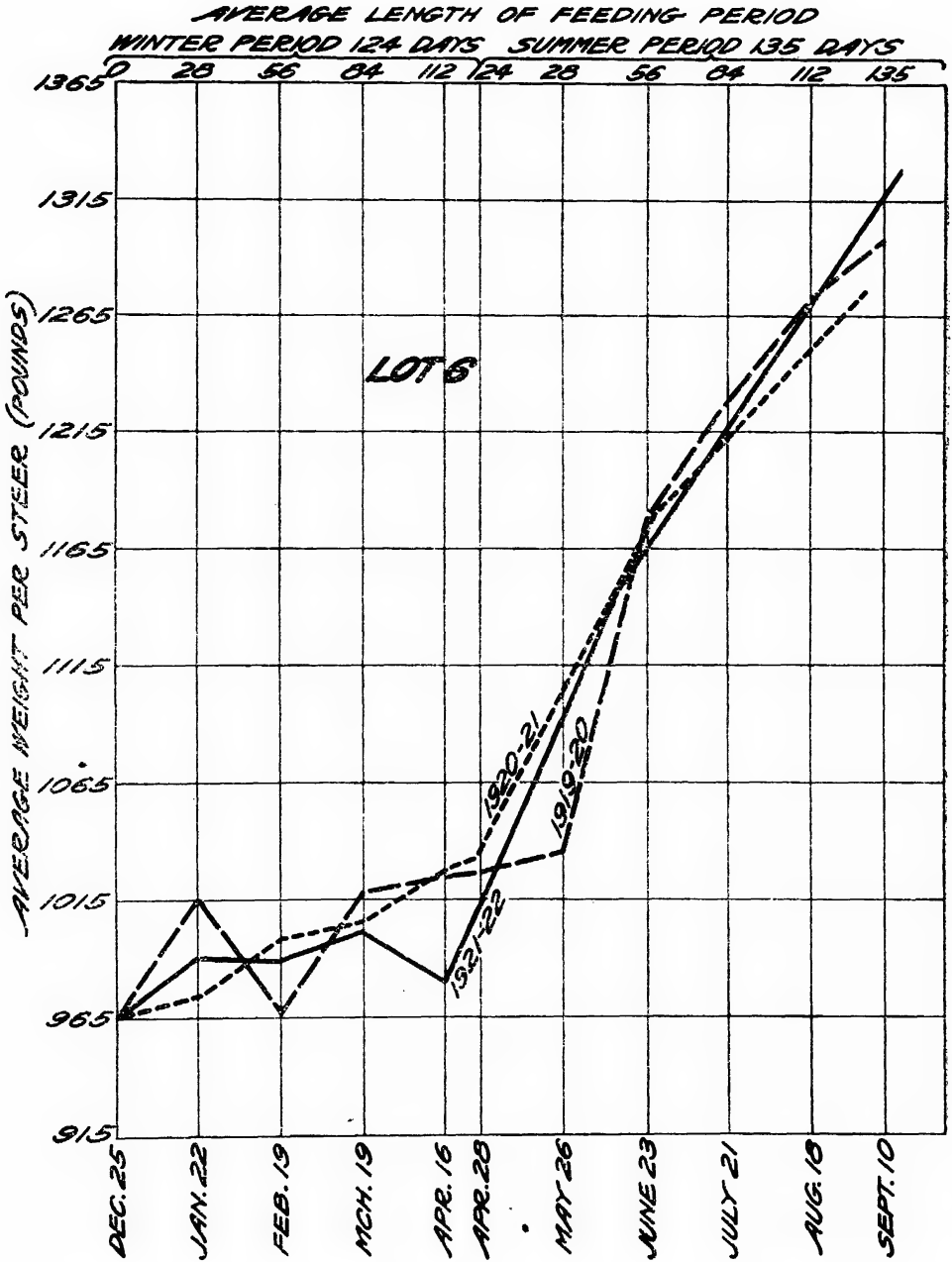


FIG. 7.—Results of winter and summer (grass) feeding for Lot 6. These steers were fed the following average ration during the winter: Corn silage, 24.8 pounds; mixed hay, 7.3 pounds

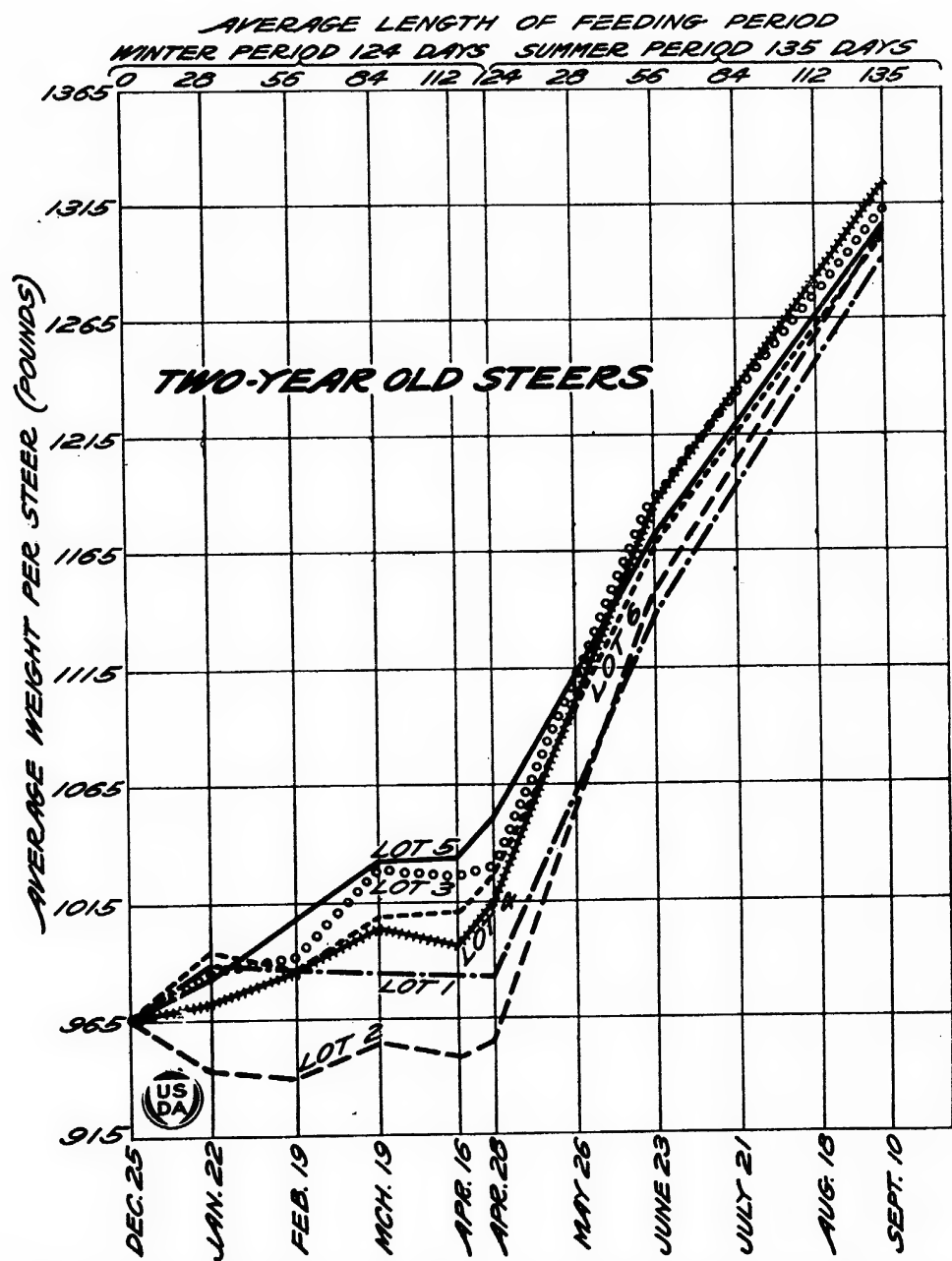


FIG. 8.—Average results of three years' winter and summer (grass) feeding for the six lots of steers



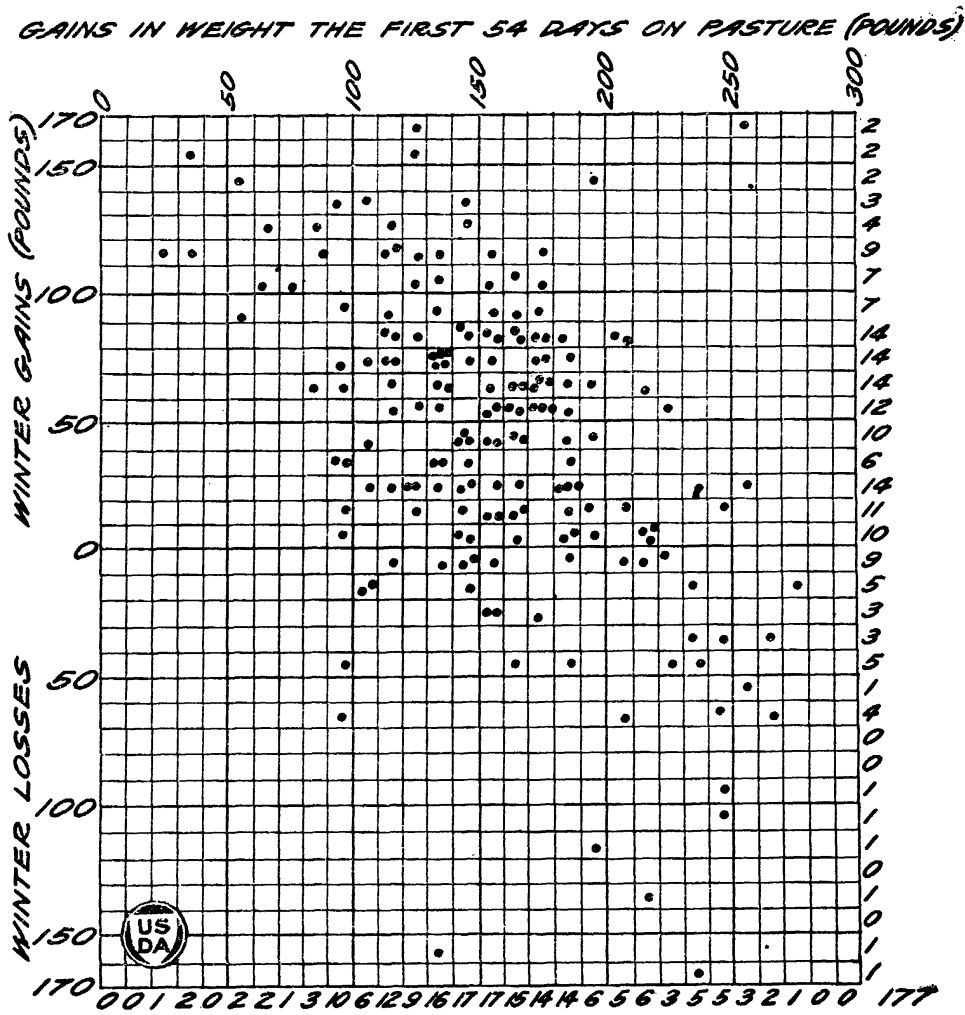


FIG. 9.—Correlation between the winter losses and gains and the gains for the first 54 days (average for three years) on pasture, based on the individual steers

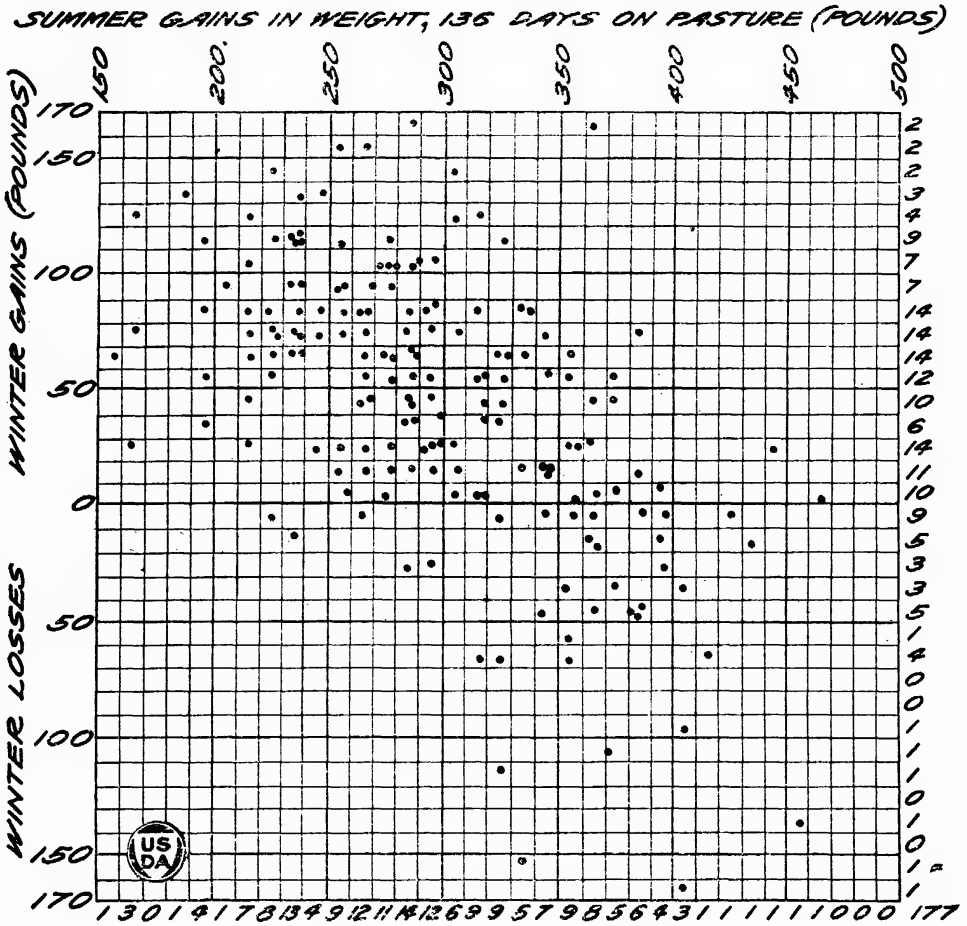


FIG. 10.—Correlation between the winter losses and gains and the gains on pasture the following summer based on the individual steers.

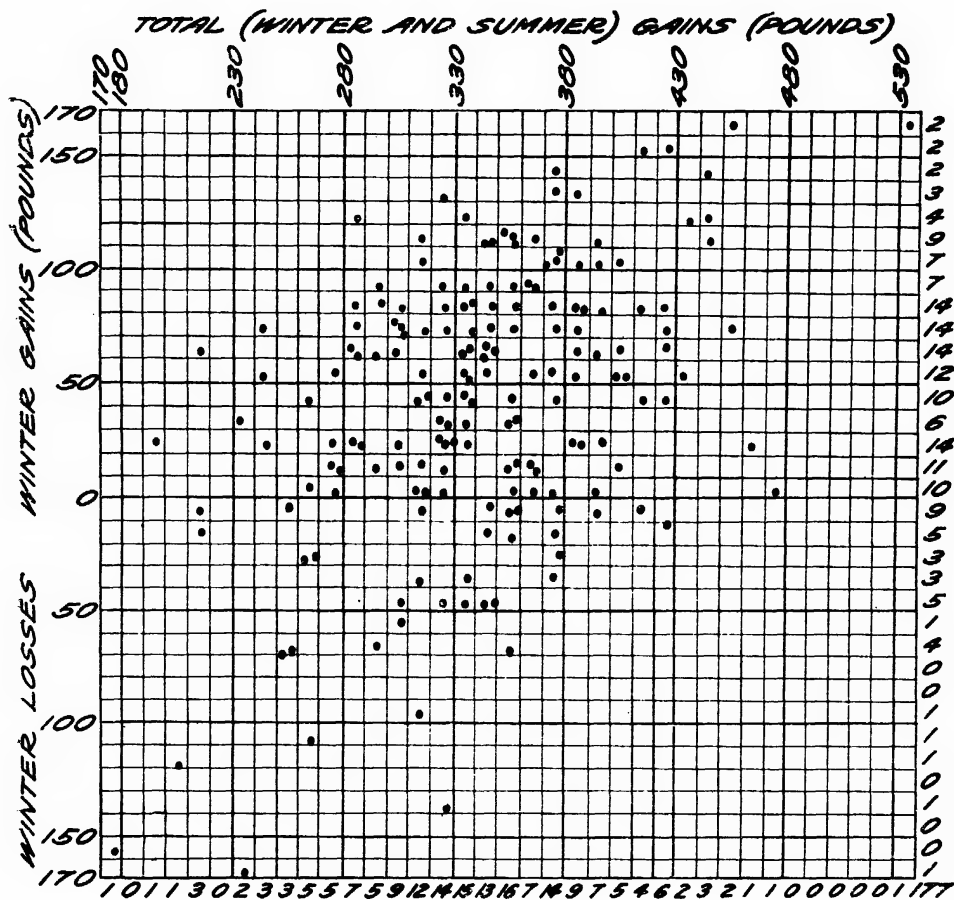


FIG. 11.—Correlation between the winter losses and gains and the total gains for winter and summer periods, based on the individual steers

SHRINKAGE IN TRANSIT AND DRESSING PERCENTAGE

The shrinkage in transit, commonly called "drift" in the Appalachian region, and the dressing percentages are given in Table V for the first two years. Owing to conditions incident to marketing the cattle, the data at the end of the third summer were not obtained. They were shipped either on the same day or on

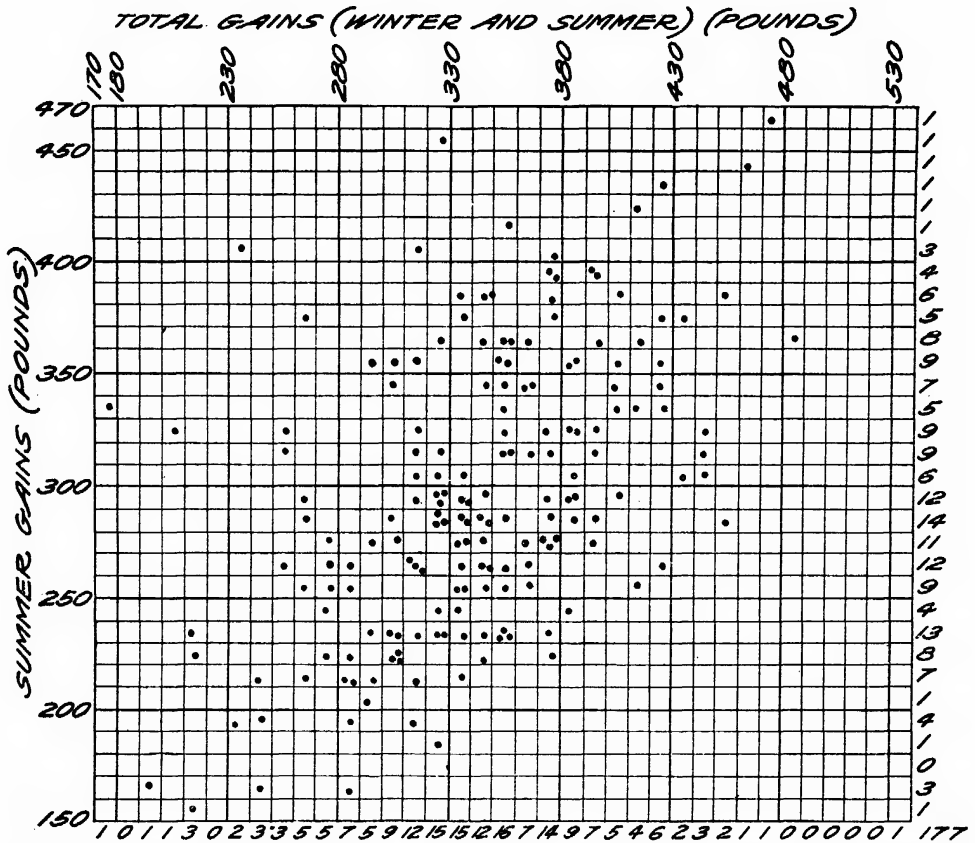


FIG. 12.—Correlation between the gains of the summer periods and the total gains for the winter and summer periods, based on the individual steers

the day after the final farm weights were taken. The trip to Jersey City usually occupied about three days, the cattle being unloaded in transit once for feed and water. They were sold and slaughtered soon after reaching market. Variations in shrinkage and dressing percentage of the several lots are not considered to be sufficiently large for drawing conclusions relative to the methods of winter feeding. However, it should be noted that Lot No. 4, which made the largest gain for the year, dressed highest, and that Lot No. 1, which made the least gain, dressed lowest.

TABLE V.—*Market weight, shrinkage in transit, and dressing percentage per steer for the first and second years' work*

Lot No	Winter feed	Season	Market weight	Shrinkage, or "drift," in transit	Percentage of drift	Dressed weight	Farm weight dressing percentage	Market weight dressing percentage
			<i>Pounds</i>	<i>Pounds</i>	<i>Per cent</i>	<i>Pounds</i>	<i>Per cent</i>	<i>Per cent</i>
1	Mixed hay and ear corn.....	1919-20	1,203	66	5.2	673	53.0	55.9
		1920-21	1,244	77	5.8	687	52.0	55.2
		Average.....	1,223	71	5.5	680	52.5	55.6
2	Corn silage.....	1919-20	1,174	62	5.0	668	54.0	56.9
		1920-21	1,202	88	6.8	685	53.1	57.0
		Average.....	1,189	75	5.9	676	53.5	56.9
3	Corn silage.....	1919-20	1,187	59	4.7	677	54.3	57.0
		1920-21	1,246	92	6.9	709	53.0	56.9
		Average.....	1,216	75	5.8	693	53.6	57.0
4	Corn silage and cottonseed meal.....	1919-20	1,225	66	5.1	704	54.5	57.4
		1920-21	1,229	85	6.5	699	53.2	56.9
		Average.....	1,227	75	5.8	701	53.8	57.1
5	Corn silage, wheat straw, and cottonseed meal.	1919-20	1,223	47	3.7	681	53.6	55.7
		1920-21	1,215	79	6.1	694	53.6	57.1
		Average.....	1,220	61	4.8	687	53.6	56.3
6	Corn silage and mixed hay.....	1919-20	1,187	77	6.1	679	53.7	57.2
		1920-21	1,211	84	6.5	684	52.8	56.5
		Average.....	1,199	80	6.3	681	53.2	56.8

## CONCLUSIONS

On the whole, the steers which made larger winter gains also made larger total gains for the year when fattened on grass the following summer (correlation, 0.43).

The steers which made only slight gains or lost weight during the winter made greater summer gains on pasture than steers which made larger winter gains (correlation between winter and summer gains, -0.57).

Consequently, differences in weight of steers at the end of the winter, due to rations fed, are gradually minimized during the time of summer fattening on grass. An advantage of 100 pounds at the end of winter falls to one of only 41 pounds after 136 days on grass.

Since differences in weight due to winter feeding are gradually minimized, but not wholly overcome, during the summer season of fattening on grass, it is important that cattle to be marketed early should gain considerably more weight during the winter than if they are to be marketed late. An advantage of 100 pounds at the end of winter falls to one of 62 pounds after 54 days on grass.

There is so little difference between the gains made by the steers in the different lots at the end of the summer grazing period that any conclusion as to the best winter ration must take into consideration the cost of the ration.

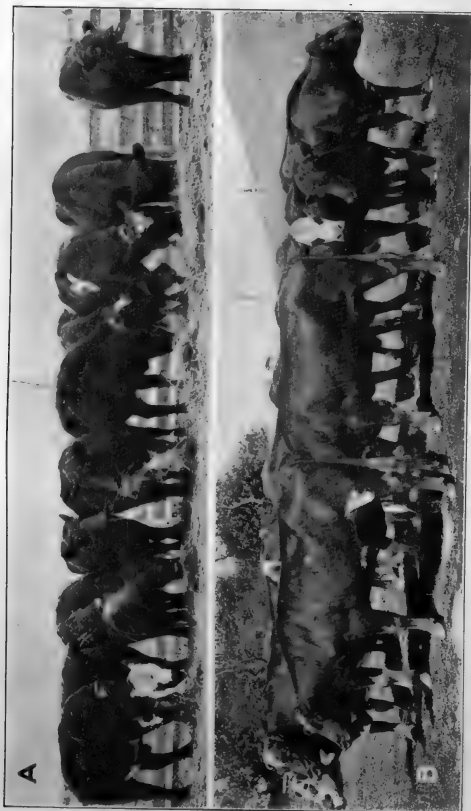
Succulent rations of silage alone, or silage, cottonseed meal, and straw, or silage and mixed hay, as used in this experiment, are cheaper and produce greater gains for the year than a dry ration of mixed hay and ear corn.

**PLATE 1**

**A.—Lot 1 at the beginning of the third year's work; winter ration, mixed hay, 15 pounds; ear corn, 2 pounds.**

**B.—Lot 1 at the end of the third year's work; average gain per steer, 307 pounds .**

**(1232)**







**PLATE 2**

**A.—Lot 2 at the beginning of the third year's work; winter ration, silage, 28 pounds.**

**B.—Lot 2 at the end of the third year's work; average gain per steer, 378 pounds.**

### PLATE 3

A.—Lot 3 at the beginning of the third year's work; winter ration, silage, 36 pounds.

B.—Lot 3 at the end of the third year's work; average gain per steer, 378 pounds.





#### PLATE 4

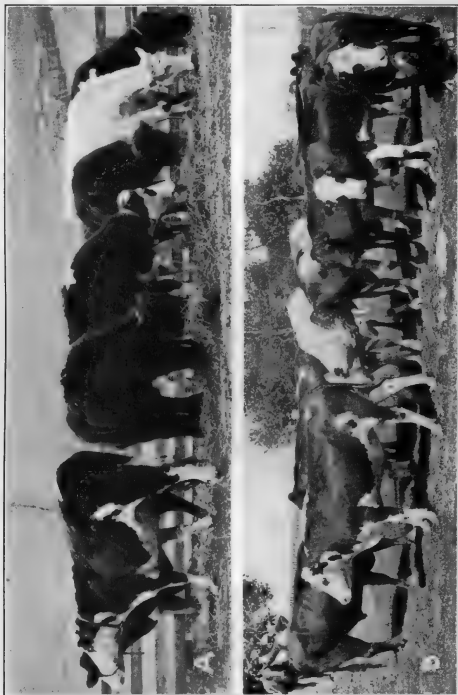
A.—Lot 4 at the beginning of the third year's work; winter ration, silage 30 pounds; cottonseed meal, 1.5 pounds.

B.—Lot 4 at the end of the third year's work; average gain per steer, 391 pounds.

## PLATE 5

Lot 5 at the end of the first winter feeding period, April 22, 1920. They represent the type of steers used in the first year's work. Aberdeen Angus breeding predominated.







## PLATE 6

A.—Lot 5 at the beginning of the third year's work; winter rations, silage, 25 pounds; straw, 6 pounds; cottonseed meal, 1 pound.

B.—Lot 5 at the end of the third year's work; average gain per steer, 377 pounds.

## PLATE 7

A.—Lot 6 at the beginning of the third year's work; winter ration, silage 25 pounds; mixed hay, 6 pounds.

B.—Lot 6 at the end of the third year's work; average gain per steer, 362 pounds.





PLATE 8

The six lots of steers in September, 1922, at the lower corner of their pasture. They are about to be driven 5 miles to be loaded and shipped to Jersey City.

## PLATE 9

Looking from Muddy Creek Mountain across a branch of the Greenbrier Valley. The steers were fattened on a pasture at the foot of the mountains in the upper part of the picture.



# A STUDY OF DOUGLAS FIR REPRODUCTION UNDER VARIOUS CUTTING METHODS <sup>1</sup>

By JACOB ROESER, Jr., *Forest Examiner, Fremont Forest Experiment Station, Forest Service, United States Department of Agriculture*

In the fall of 1913, four plots, each 200 by 200 feet, and subdivided into 20-foot sections, were laid out in a typical Douglas fir (*Pseudotsuga taxifolia*) stand in the vicinity of the Fremont Forest Experiment Station, on a north exposure, with a fairly uniform slope of approximately 35 per cent and an elevation of 9,100 feet. The merchantable stand was approximately 265 years old and consisted of Douglas fir and Engelmann spruce (*Picea engelmanni*) in mixture with some limber pine (*Pinus flexilis*), western yellow pine (*P. ponderosa*), and aspen (*Populus tremuloides*). Approximately 78 per cent of the larger-sized trees, over 6 inches in diameter, were Douglas fir, while only 29 per cent of the smaller trees were of this species, indicating a gradual replacement of the fir by the spruce and a general tendency toward an ultimate spruce type. The soil on the plots is a granitic gravel.

The four plots, arranged from west to east and separated by 40-foot isolation strips, were prepared as follows:

No. 1 was clear cut; No. 2 was left in its original condition to serve as a control, with 1,145 trees of Douglas fir, Engelmann spruce, limber and yellow pine, aspen, and willow (*Salix sp.*) of all ages comprising the stand; No. 3 was cut under the shelterwood plan, removing all but 83 of the thriftiest mature Douglas firs, which were well scattered over the area and in 1916 averaged 9.95 inches in diameter breast high; while plot No. 4 was cut as nearly under a selection system as the condition of the stand would permit. Two hundred and seventy-one Douglas firs were left, representing all sizes from 4½ feet high to mature trees. While the original stand was by no means even-sized, it was to a large extent even-aged, and many, if not most, of the small trees left were merely small through long suppression.

Following the cutting operation, all reproduction below 4½ feet in height was removed so as not to interfere with future counts.

In order to study the effect of different methods of slash disposal upon the character of the reproduction secured, the slash left after logging on the eastern half of each of the three cut-over plots was scattered over the ground, while that on the western half was piled and burned.

The first reproduction count was made in the year 1915, but was only partially completed because of the advent of bad weather. A complete count was made in 1916. No counts were made in 1917 and 1918, although casual observations during 1917 showed no new germination had come in, and that most of the 1916 germination had disappeared.

In the summer of 1919, a fairly complete study, including reproduction counts, tree measurements, the relation of brush, humus depth, grass, and root length to reproduction and survival, and observation of climatological conditions, was made and reported upon, and every fall since then reproduction counts have been made on 22 of the 20-by-20-foot sections located in diagonal strips across each plot, running S. 45° W. up the slope, and representing an average of all surface

<sup>1</sup> Received for publication June 19, 1924—issued Nov., 1924.



conditions. The actual area of each group of sections representing a certain method of treatment is 0.202 acre.

The total number of seedlings of the coniferous species present at each of the last 5 counts, on the basis of one acre, was as follows:

	Clear-cut	Control	Shelterwood	Selection
1919.....	1,287	9,009	4,035	3,352
1920.....	579	2,192	4,633	3,980
1921.....	1,083	3,826	7,028	6,465
1922.....	1,267	2,901	10,316	8,530
1923.....	1,569	3,821	13,427	9,707

It will be seen that since 1919 the selection and shelterwood areas have tripled the amount of reproduction, and that the clear-cut area is building up slowly, while the area under the original forest is showing no net increase. The extremely large number of seedlings present upon the control plot in 1919 was due to an exceptionally heavy crop of 1918 Engelmann spruce seedlings. Because of the presence of spruce seed trees upon the control plot this species is always well represented in the new crop of seedlings, considerably more so than on any other plot. In 1923, 42 per cent of the new control crop was spruce, while on the shelterwood and selection plots this species was present only to the extent of 6 and 2 per cent, respectively, and no new spruce seedlings at all were found upon the clear-cut area.

In each count the seedlings were tallied as new, 1 year old, 2 years old, and 3 years and older. The last class is considered established and is referred to as the "surviving" class. From a study of conditions on plot No. 3 in 1919, only approximately 6 per cent of the fir seedlings live after the first year. One-half of these die during the following three years, and more than likely most of them during the second year. The mortality rate after the third year is low.

### SURVIVAL OF SEEDLINGS

Although it is always desirable to secure copious reproduction, the criterion of success for any system is the amount of survival, and it is in this respect that the virgin stand falls down. Fifty-nine per cent of all the reproduction present in 1923 on the control plot was represented by 1923 seedlings, of which the expectation of survival is very low. Under the shelterwood cutting all but 6 per cent of the trees were at least one year old, as was the case also on the clear-cut area, while in the selection forest only 1 per cent were new seedlings.

The following summary gives the number of established, or three-year-old, seedlings per acre for each system:

	Clear-cut	Control	Shelterwood	Selection
1919.....	287	238	811	480
1920.....	213	233	1,127	550
1921.....	881	2,271	5,094	3,985
1922.....	900	1,430	6,436	4,592
1923.....	1,005	1,237	6,891	4,638

Again, as in the case of total germination, there is a perceptible yearly increase on the cut-over plots. The big increase in 1921 is due to the 1918 Douglas fir seedling crop passing into the survival class. Under the virgin forest, the 3-year-old class is gradually decreasing, indicating that the seedlings are unable, even

after apparently becoming established, to combat the natural conditions under which they must grow.

It was found that only 32 per cent of the trees on the control plot in the 1923 count were 3 years and older, while on the selection plot these reached 48 per cent. Upon the shelterwood plot 51 per cent were 3-year seedlings, and 64 per cent upon the clear-cut plot.

The different species were represented in the 1923 surviving class by the following percentages:

	Clear-cut	Control	Shelterwood	Selection
Douglas fir.....	62	57	80.3	85
Engelmann spruce.....	20	36.5	19.2	12.7
Limber pine.....	18	6.5	0.4	2.1
Yellow pine.....	0	0	0.1	0.2

One object of the cutting experiment was to secure a young stand of Douglas fir to the exclusion of other species and, as will be seen, the shelterwood and selection plots have come closest to securing a fulfillment of this object. To a large extent, success in securing reproduction is dependent upon the seed supply, which has been abundant on all plots but the clear-cut. The high percentage of survival that this area shows indicates not so much growing conditions superior to the other areas as that little new reproduction is coming in. What does appear is mainly confined to the west (windward) and south sides of the plot, adjacent to heavy timber. It is evident that 1 acre is too large an area to be successfully reseeded from the sides when in the form of a square.

In general, the humus on the clear-cut plot is too deep to favor new reproduction, and an accumulation of new humus in the form of aspen leaves is beginning to cover the soil with a layer which seedling roots find it difficult to penetrate. The entire area is now covered with a heavy stand of aspen, some of which is 15 feet in height. While this is not so thick as through shade and moisture competition to preclude the possibility of fir reproduction, it is gradually building up such a barrier upon the ground as to offer serious hindrance to any reproduction that might appear despite the lack of seed.

The selection plot ranks low in percentage of spruce reproduction because it is the only treated plot having no spruce seed trees on its windward side. In general it has furnished as much fir reproduction as has the shelterwood plot, but it does not stand as high in survival of this species. It is quite possible that this is due to more favorable location of the shelterwood plot, as will be shown; but natural conditions of growth also favor the shelterwood plan in stands like this that are mostly even-aged.

### MOISTURE A DETERMINING FACTOR

The results of several years' investigation of climatic factors upon the plots lead to the following interesting deduction regarding moisture supply: During the early part of the year moisture conditions are most favorable in the old-growth stand, where wind movement and insolation, the chief factors in evaporation, are at a minimum. The selection and then the shelterwood areas follow in order. Because of its exposure, the clear-cut plot shows the lowest percentage of soil moisture for the whole season. Since soil moisture appears to be a controlling factor in Douglas fir reproduction, any considerable amount of early germination on the clear-cut area is made difficult. However, there is less seasonal variation in moisture here and a more even supply than under any of the

other systems of treatment, on account of the small amount of tree growth. Thus it is that seedlings that get a start while stored-up moisture is available in the spring, and before the aspen activity starts, are furnished with a fairly uniform supply of moisture throughout the season and survive. This is in contrast particularly to the condition under the original stand, where the multitude of seedlings, accustomed at first to plentiful moisture, hence not stimulated to deep rooting, easily succumb as this moisture is rapidly exhausted by the roots of the older trees.

The shelterwood area, being protected by the control plot against excessive wind movement and evaporation, has practically as plentiful a supply of moisture in the spring as the selection plot with more crowns. This is due also to the accumulation of drifted snow in the winter along the west side, and a relatively small drain by the old trees, and insures good germination and survival. The factor of winter protection, provided by drifting snow and shelter from the control plot, is, however, a matter of relative position rather than one of the silvicultural systems involved. The prevention of drifting, on the other hand, especially on the clear-cut area, is a strong argument in favor of brush scattering.

But the moisture supply or lack of it, which is the determining factor in the success of reproduction, may in turn be determined by density of stand. The percentage of seedlings surviving is governed most directly by late summer or fall drought, which is most severe where the heaviest stand remains, in spite of the fact that the degree of protection of soil and seedlings varies with the density of the stand. All other conditions are inconsequential in comparison with the drought caused by the drain of the older trees upon the moisture near the surface. The seedling's worst enemy in many cases may be its parent tree.

The importance of soil moisture in the success of Douglas fir reproduction upon these plots was first brought out by F. B. Notestein in 1912. He established sixty 10-by-10-foot reproduction plots, regularly scattered over the entire area before it was cut, and studied the influence of light intensity, soil moisture at 1, 2, and 6 inches, organic content of the soil at 1 and 2 inches, and soil temperature at 6 inches, upon the character of the reproduction.

It was determined that Douglas fir will reproduce successfully in a light intensity of 8.5 per cent. In a stand having an average light intensity of 27.24 per cent, reproduction averaged most abundant at points of least light; but Notestein was satisfied this simply meant that there was sufficient light everywhere under such a stand, which is comparable to the present control stand, but that in the openings where light is intense other conditions were unfavorable.

Soil moisture was found to decrease with increase of light. At the same time, Douglas fir reproduction was greatest at those points having the greatest soil moisture. All facts indicated that soil moisture not only is a more potent factor than light in controlling reproduction, but that it is the controlling factor.

The total soil moisture varied directly with the amount of organic matter in the soil, while the available soil moisture varied inversely with it. The relation between the amount of humus and reproduction is uncertain, but the facts brought out indicate the probability that there is a point up to which humus increases water capacity more than wilting coefficient, and is, therefore, favorable to reproduction, and beyond which it has the reverse effect.

No relation was found between soil temperature and reproduction.

## SLASH SCATTERING VERSUS SLASH BURNING

In order to compare the relative values of scattering slash and piling and burning it, the total Douglas fir, Engelmann spruce, and limber pine seedlings present in 1923 following the two methods of slash disposal are shown in Table I.

TABLE I.—*Effect of slash disposal on seedlings of various ages and species under different cutting systems*

Slash disposal and cutting methods	Number of seedlings remaining in 1923, by species and year of origin											
	Douglas fir				Engelmann spruce				Limber pine			
	1923	1922	1921	Older	1923	1922	1921	Older	1923	1922	1921	Older
Slash scattered:												
Shelterwood.....	901	267	5,465	5,009	30	20	277	525	00	40	0	30
Selection.....	1,277	297	2,455	4,287	50	0	89	356	20	20	0	129
Clear-cut.....	59	10	178	396	0	10	59	178	20	89	30	59
Total.....	2,237	574	8,098	9,692	80	30	425	1,059	40	149	30	218
Slash burned:												
Shelterwood.....	505	178	4,900	6,069	59	79	624	2,119	20	40	69	20
Selection.....	871	346	4,613	3,594	0	0	59	822	0	10	30	69
Clear-cut.....	109	20	446	851	0	0	30	228	10	20	40	297
Total.....	1,485	544	9,959	10,514	59	79	713	3,169	30	70	139	386

TABLE II.—*Effect of slash disposal upon all seedlings, all methods of cutting combined, expressed in percentage of survival*

Method of slash disposal and year of count	Percentage of survival of all seedlings by species			Percentage of survival of established seedlings (3 years old and over) by species		
	Douglas fir	Engelmann spruce	Limber pine	Douglas fir	Engelmann spruce	Limber pine
Slash scattered:						
1923 survival.....	48	28	41	48	25	36
1922 survival.....	48	28	38	48	25	33
1919 survival.....	51	28	35			
Slash piled and burned:						
1923 survival.....	52	72	59	52	75	64
1922 survival.....	52	72	62	52	75	67
1919 survival.....	49	72	65			

Since Douglas fir is the principal species and includes the bulk of the reproduction it may be said that the method of slash disposal plays a minor part in germination and survival. The results for all the counts so studied are very consistent. The fir, if anything, shows a very slight preference for the areas from which the slash has been removed by fire.

Engelmann spruce reveals a decided preference for slash-burnt areas, and a greater numerical survival there. This condition can be attributed largely to the fact that the cleared areas are more favorably located with reference to spruce seed sources. On a percentage basis this species survives somewhat better under slash, once it gets a start. The 1916 crop had 6.2 per cent surviving in the open in 1919, and 15.4 per cent under slash. This ratio may not be strictly representative, for the majority of years, but is credible in view of the rather slow rooting of spruce, its tolerance to shade, and a sensitiveness to high temperatures, which equals though it does not exceed the sensitiveness of Douglas fir.

Limber pine exhibits a preference for the open both for germination and for survival, which is in keeping with the tree's general demand for open conditions, and its habit of thriving in exposed locations.

Summarizing it may be said for all species that the method of slash disposal plays a minor part in germination and survival. Nor can the amount of slash scattered over the area be considered a criterion of the amount of immediate protection offered by logs, stumps, juniper, grass, twigs, and small litter, which furnish the seedlings protection through the critical period of the third year, when there is greatest danger from direct insolation. Again, although scattered slash effectively prevents grasses coming in after opening up the stand, nothing discovered in this study has indicated that grasses are detrimental to fir reproduction; on the contrary, it has been found that the seedlings prefer to a surprising extent the protection offered by grasses. The number of survivors found and their general thrift indicate that the losses here are no greater than under slash.

In 1919, 85 per cent of fir and 80 per cent of spruce had come up under immediate protection, irrespective of cleared and slash areas; in 1922 these figures were 87 and 82 per cent, respectively. Evidently fir takes advantage of cover more than spruce does. Healthy 1917 and 1918 fir seedlings were more abundant under slash in 1919, while healthy spruce of the same age was more abundant in the open. This is attributed to the fact that the humus under slash is a trifle too deep for spruce. However, both species show the best thrift under protection, as defined above. As a whole, the seedlings naturally had more chance to come up near protection upon areas where the slash had been scattered, but the difference was not a great one.

HUMUS DEPTH AND ROOT LENGTH

Though the principal factor in germination will be found to be moisture supply, survival is dependent to a great extent upon humus depth. There exists a definite relationship between drought and depth of humus and also between tolerable depth of humus and immediate protection. Protection permits about one-half inch of additional humus for fir, and less for spruce. A protected seedling may survive the first and crucial season in humus in which an unprotected one would succumb.

Since humus depth is important in governing the distribution and survival of fir and spruce seedlings, root length is of prime importance. The root must be able to push through the comparatively open, quickly desiccated layer of humus and secure a hold in the mineral soil beneath, where, if the seedling is to survive its first drought period, it must depend upon a more or less steady moisture supply.

A study of root length in relation to humus depth was made in 1919. Only a limited number of trees were studied, but enough, it is believed, to reflect the general trend of the relationship. The following figures represent seedlings about one year old:

DOUGLAS FIR

	Average root length	Average depth of humus
	<i>Inches</i>	<i>Inches</i>
Vigorous.....	3.94	0.81
Wilted.....	2.74	1.58
Dead.....	2.82	2.40

Average root length 2.75 inches.

ENGELMANN SPRUCE

Vigorous.....	2.75	1.18
Wilted.....	2.08	1.04
Dead.....	1.51	1.71

Average root length 1.80 inches.

Fir seedlings wilt with a difference of 1.16 inches in root length over humus depth, while the difference between vigorous seedling roots and humus depth is 3.13 inches. This would indicate a maximum depth of humus of between 1.50 and 1.75 inches for vigorous fir seedlings under protection, and 1 to 1.25 inches in the open. Spruce seedlings wilt with a difference of approximately 1.04 inches in root length, the difference for vigorous seedlings being 1.57 inches. Thus the maximum humus depth for vigorous seedlings will be about 1.4 inches. Without protection this figure will be about 1.1 inches.

The average humus depth in inches over the plots in 1919 was:

	Slash scattered	Slash piled and burned	Average
Clear-cut.....	1.90	1.20	1.55
Original forest.....			1.18
Shelterwood.....	1.30	.75	1.02
Selection.....	1.25	.77	1.01

Except upon the slash-covered area under clear cutting, in no instance was the humus too deep for fir, and the same may be said for spruce where offered a modicum of protection, although the margin in most instances was smaller. Clear cutting in general is not desirable, considered from the standpoint of reproduction. Under the original stand, although the margin for healthy spruce seedling development is rather small, the long-term history of the stand indicates that spruce reproduction is actually favored over fir. With the great number of spruce seed germinating in virgin stands, it is quite possible that enough of these find favorable humus conditions to provide replacement, and that other conditions, such as lack of immediate protection and lack of moisture, act unfavorably upon the fir.

#### HEIGHT AND PROTECTION CORRELATED

When all species were grouped as protected and unprotected, it was found that protected seedlings are uniformly taller than those unprotected, this being more true of fir than of spruce. The average difference for fir is 0.27 inch, while for the spruce it is only 0.02 inch; but this latter figure is misleading due to an apparent reversal of form of the selection seedlings, which were 0.45 inch taller when unprotected. In every instance, the humus is from 0.3 to 0.6 inch deeper for protected fir and spruce seedlings. Although no measurements of root lengths were made, it is hardly likely that humus depth has any influence upon survival after the third year. However, assuming that the humus has increased in depth only a little since the advent of the seedlings, the favorable effect of protection combined with relatively shallow humus is evident.

It is not known at what rate humus has accumulated upon the plots since the original measurements were made, although it is reasonably certain that the heavy stand of aspen upon the clear-cut plot is having the effect of building up the humus layer and making it more difficult for seedlings to get started. This may also be the case upon the scattered-slash areas of the selection and shelterwood plots, but the young stand which has already established itself here makes it unnecessary to give any more consideration to the character of the humus.

An effort has been made in figures 1, 2, and 3 to illustrate the trend of height growths for Douglas fir, Engelmann spruce, and limber pine seedlings beginning with the fourth year, under the various methods of cutting and under the original stand. Insufficient data were at hand to construct a curve for limber pine on the shelterwood area, but the curves that are shown emphasize the superior per-

GROWTH OF DOUGLAS FIR SEEDLINGS  
UNDER VARIOUS SILVICULTURAL METHODS AND IN THE  
ORIGINAL FOREST IN DOUGLAS FIR STANDS

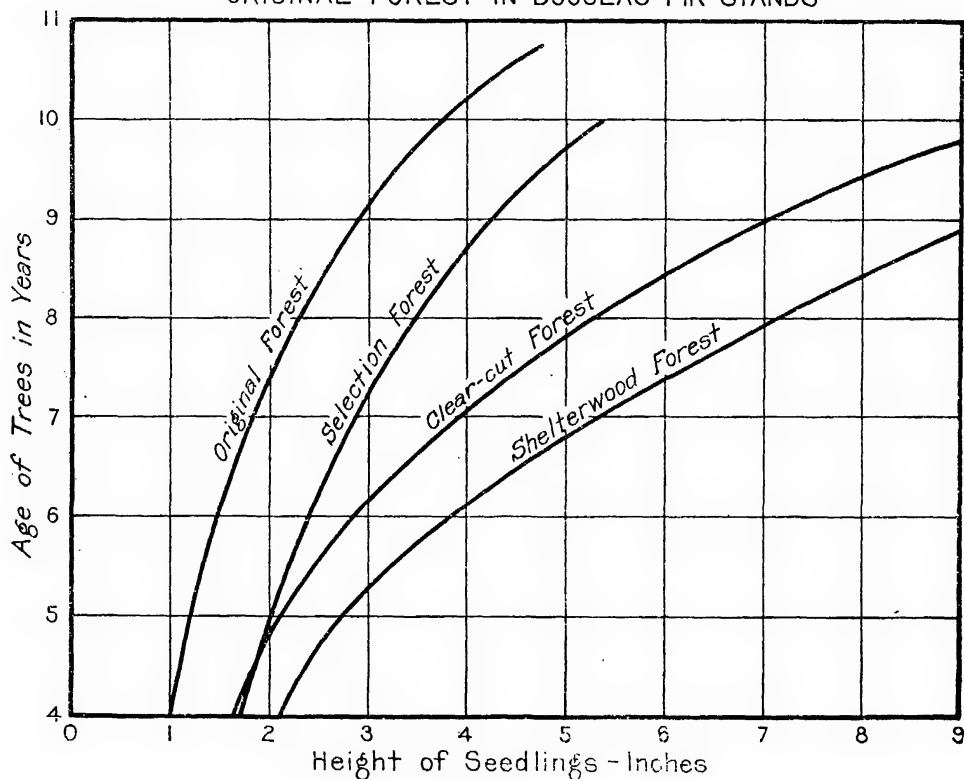


FIG. 1

GROWTH OF ENGELMANN SPRUCE SEEDLINGS  
UNDER VARIOUS SILVICULTURAL METHODS AND IN  
THE ORIGINAL FOREST IN DOUGLAS FIR STANDS

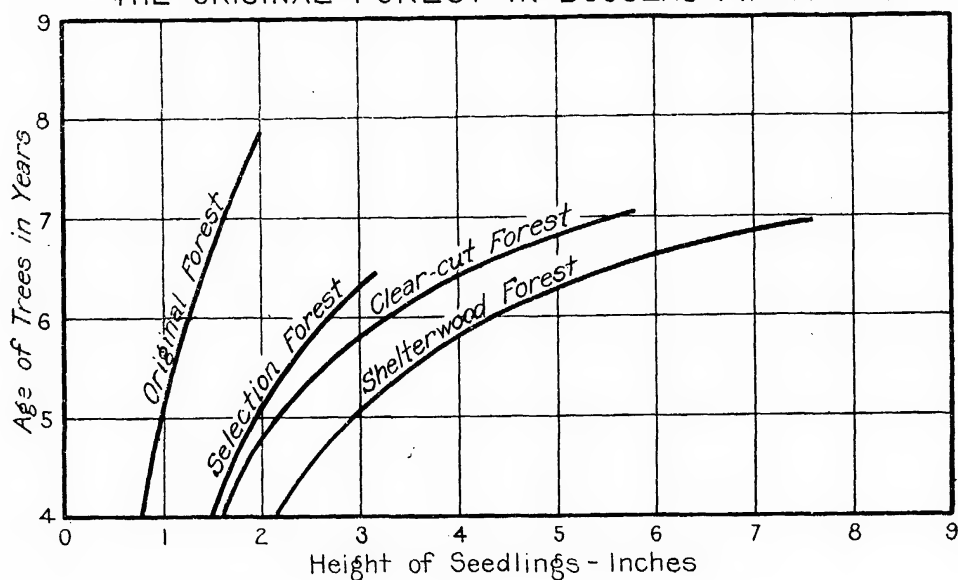


FIG. 2

formance of Douglas fir and Engelmann spruce seedlings upon the shelterwood plot. In every instance, the clear-cut, selection, and original stands rank in order downward for each species.

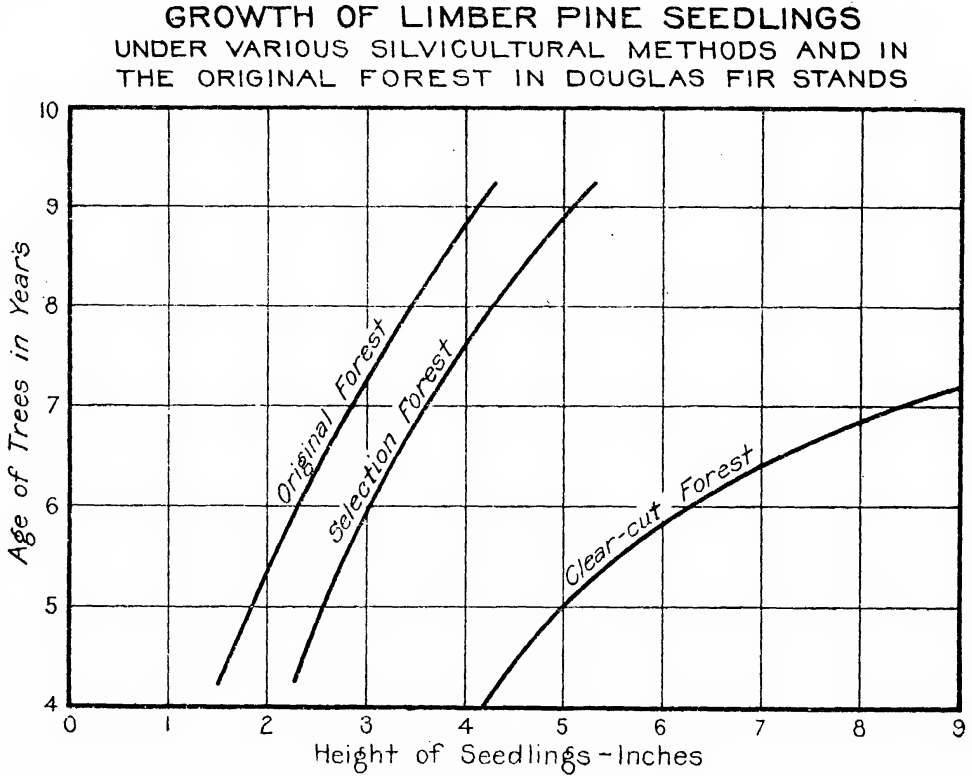


FIG. 3

### SUPERIORITY OF SHELTERWOOD

For the past few years, the most striking fact noted in connection with the reproduction upon the plots has been the superior development of the young trees under the shelterwood cutting. This good growth is not confined to any one section or sector, but characterizes the young trees of both Douglas fir and Engelmann spruce over the entire area. It is rather difficult to place the responsibility for this better development upon any of the conditions which establish this plot as superior to the selection plot. In order to determine the relation between growth rates under the various methods of treatment, the seedlings on at least one half of the sections of each plot and all of them upon the clear-cut plot were measured in 1923. At the same time, the depth of humus was measured as near each seedling as could be done without disturbing the young tree, and the age of each tree was also determined.

On both slash-scattered and slash-burned areas under the shelterwood cutting, the Douglas fir seedlings averaged larger than on any of the other plots. Upon the slash-scattered area their average height was 3.53 inches, as against 2.66 inches for the clear-cut area, and 2.15 inches for the selection. Upon the slash-burned area, the respective heights in the same order were 3.12, 2.70, and 2.70 inches. The average height of fir seedlings under the old stand was but 1.49 inches. These figures represent groups varying from 40 to 341 seedlings, with average ages varying from 5.40 to 6.05 years and uniformly greater upon the slash-scattered areas. Curiously, the seedlings under slash on the shelterwood plot were larger than those under piling and burning, which was not the case with the selection



and clear-cutting systems. Humus depth, which does not represent the average depth to be encountered upon the various plots, and, therefore, can not be compared with previous measurements, was least for the "clear-cut" seedlings, which averaged next to the "shelterwood" seedlings in height, and was greatest for the old stand. Except for the latter plot where the humus depth was 1.93 inches, the depths at the points where the seedlings are growing (1.30 inches upon the cleared areas and 1.65 inches under brush) are fairly uniform, and suggest, perhaps, the most favorable depth for fir survival. The average, also uniform difference between the depths of humus under the two methods of slash disposal, was 0.35 inches, with scattering naturally resulting in the deeper humus.

The variation in ages was also fairly small, the average age of the surviving seedlings, or those over 3 years, ranging from 5.52 years for the shelterwood to 5.74 years for the virgin stand.

Spruce shows a reaction similar to fir in the shelterwood cutting, but the "selection" seedlings are somewhat larger than those upon the clear-cut plot. Average heights upon the shelterwood area were 3.26 inches with slash burning and 3.21 inches under scattered slash; upon the clear-cut area these heights were 2.13 and 2.18 inches, and upon the selection 2.78 and 1.79 inches respectively. If the results are correctly interpreted, slash burning has a beneficial effect. Spruce here again shows its preference for open conditions and shallower humus. The average height of this species for five-year seedlings in the original stand is only 0.97 inches, whereas the fir seedlings are 1.49 inches tall but a year older. The low average age of spruce on this plot is typical of all plots, and indicates, in view of the relatively large number of seedlings which start, that this species is being surpassed by the fir, contradictory though this may appear to the common idea of what is actually taking place. The difference in average ages upon the other plots was slightly lower than under the original stand, varying from 0.4 to 0.7 years.

Limber pine seedlings are much larger upon the shelterwood (6.60 inches under brush and 6.40 inches in the open) and clear-cut areas (7.63 and 6.95 inches) than upon the others (3.15 and 5.22 inches for the selection and 2.56 inches under the original forest), but show no decided preference for either method of slash disposal. The average age of these trees, 6½ years, shows that little new reproduction of this species is coming in. Practically all of the reproduction can be traced to seed originally stored in the duff or cached by squirrels.

This study of reproduction under the various cutting methods in Douglas fir stands has brought out the desirability of the shelterwood system, as particularly adapted to Douglas fir stands. It has not proved that slash scattering, employed mainly to furnish shade for the seedlings and help conserve soil moisture, has any decided silvicultural advantages over piling and burning of slash, save that it is the less expensive and therefore more desirable method. This is more or less a question to be decided upon the ground. The natural debris resulting from logging operations should ordinarily furnish all the protection the seedlings will need.

The success secured in regenerating under shelterwood and to a great extent under selection cutting is so obvious that it is now planned to make the second cut on the shelterwood stand within the next two years. Periodic reproduction counts will no doubt be continued, to show the effect of this final cutting and the course of development in the other plots.

**PLATE 1.**

**Shelterwood cutting, looking west through center of plot toward the uncut stand in control plot.**



# THE ISOLATION AND IDENTIFICATION OF QUERCETIN FROM APPLE PEELS<sup>1</sup>

By CHARLES E. SANDO

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Some time ago the writer's interest in possible relations between chemical constituents of the apple and storage scald in this fruit led to an investigation of the nonvolatile waxlike substances covering the epidermis.<sup>3</sup> This work has been extended to include a study of the yellow flavonol pigment which occurs in apple peels. The present paper deals with the isolation of the pigment and with its identification as quercetin, a widely distributed member of the flavonol group.

The isolation of quercetin has previously been reported from apple bark,<sup>4</sup> but no mention has hitherto been made of its occurrence in the peel of the fruit.

## PREPARATION OF QUERCETIN

The material employed in this work consisted of peels of the McIntosh apple. It was obtained as a by-product in the investigations of Power and Chesnut<sup>5</sup> on the odorous constituents of apples. In their work, the fresh parings were subjected to distillation in a current of steam. The residue from the steam-distillation was used for the preparation of the flavonol. On account of this treatment and the necessity of prolonged extraction with alcohol, in the presence of malic acid contained in the parings, the glucoside of the flavonol was not obtained.

The wet peels after steam distillation were carefully dried and coarsely ground for purposes of extraction. The extractions were carried out by using a continuous extraction apparatus based on the Soxhlet principle. With this apparatus it was possible to extract about 1 kgm. of material at each loading. The dried material was first extracted separately with petroleum ether and ether in order to remove chlorophyll, carotinoids, and waxlike substances. These extractions were followed by the use of 95 per cent alcohol, which dissolved the coloring matter. The extraction with alcohol was continued for several weeks before the last traces of flavonol pigment were removed from the peels. After evaporation to eliminate the greater portion of alcohol, hot water was added to the syrupy residue. The liquid, upon the addition of neutral lead acetate solution yielded a precipitate which contained very little of the pigment. The lead precipitate was filtered off by means of a Buchner funnel and then basic lead acetate solution added to the filtrate. The basic lead acetate precipitate, which contained most of the coloring matter, was separated by filtration and decomposed by boiling with 5 per cent sulphuric acid. After removal of lead sulphate and cooling, the acid solution was repeatedly shaken with ether in a separatory funnel. Evaporation of the ethereal liquid left a residue consisting of crude

<sup>1</sup> Received for publication May 21, 1924—issued Nov., 1924.

<sup>2</sup> In connection with this work thanks are given to Dr. Frederick B. Power for his kindness in supplying the material employed.

<sup>3</sup> SANDO, C. E. CONSTITUENTS OF THE WAX-LIKE COATING ON THE SURFACE OF THE APPLE. *Jour. Biol. Chem.* 56: 457-468. 1923.

<sup>4</sup> ROCHLEDER, F. QUERCITRIN AND QUERCETIN. *Jahresber. Fortschr. Chem.* 1867: 731-732. 1869.

<sup>5</sup> POWER, F. B., and CHESNUT, V. K. THE ODOROUS CONSTITUENTS OF APPLES. II. EVIDENCE OF THE PRESENCE OF GERANIOL. *Jour. Amer. Chem. Soc.* 44: 2938-2942. 1922.

coloring matter which weighed 2.45 gm. in the air-dried state. This quantity of impure pigment was obtained from 68.6 kgm. of fresh skins, which represented 545.7 kgm. of whole apples. After purification of the crude material only about 0.5 gm. of pure pigment remained.

### IDENTIFICATION OF QUERCETIN

Acetylation of the unidentified substance was carried out by boiling the impure pigment with anhydrous sodium acetate and acetic anhydride. The reaction mixture was poured into water and set aside over night. The product thus obtained, after several recrystallizations from 70 per cent alcohol, amounted to 1.15 gm. It consisted of a feltlike mass of colorless needles which melted at 194° to 196° C. When the substance was mixed with pure authentic penta-acetylquercetin its melting point was not altered. The substance was thus identified as penta-acetylquercetin. Combustion of the anhydrous compound dried at 160° confirmed this conclusion.

0.1489 gm. gave 0.3196 gm. CO<sub>2</sub> and 0.0531 gm. H<sub>2</sub>O.

Found: C = 58.53, H = 3.99.

Acetylquercetin, C<sub>15</sub>H<sub>5</sub>O<sub>7</sub>(C<sub>2</sub>H<sub>3</sub>O)<sub>5</sub>, requires: C = 58.57, H = 3.93.

From the penta-acetylquercetin the free pigment was regenerated by hydrolysis with a few drops of sulphuric acid in glacial acetic acid. After boiling a few minutes, the mixture was well diluted with water and set aside in the ice box for several days. The insoluble compound was collected in a weighed Gooch crucible and dried at 130°.

0.8436 gm. gave 0.4984 gm. recovered pigment.

Found: Pigment = 59.08.

Acetylquercetin, C<sub>15</sub>H<sub>5</sub>O<sub>7</sub>(C<sub>2</sub>H<sub>3</sub>O)<sub>5</sub>, requires: C<sub>15</sub>H<sub>10</sub>O<sub>7</sub> = 58.98.

The substance obtained by regeneration from its acetyl derivative responded to all the tests of pure quercetin. Crystallized from dilute alcohol, it formed a glistening mass of yellow needles which were almost insoluble in water, but readily soluble in alcohol. Solution in dilute alkalis intensified the yellow color. With alcoholic ferric chloride it gave a dark-green color. When reduced in alcoholic solution with magnesium ribbon and hydrochloric acid it developed a characteristic bright red anthocyanic color. Analysis of the substance, dried at 130°, showed that it agreed in composition with quercetin.

0.1559 gm. gave 0.3399 gm. CO<sub>2</sub> and 0.0466 gm. H<sub>2</sub>O

0.1325 gm. gave 0.2891 gm. CO<sub>2</sub> and 0.0379 gm. H<sub>2</sub>O

Found: C = 59.46, 59.50; H = 3.35, 3.21

Quercetin, C<sub>15</sub>H<sub>10</sub>O<sub>7</sub>, requires: C = 59.59, H = 3.34.

### DISCUSSION

There is some reason for assuming that quercetin in apple peels may be the chromogenic substance which is responsible for the formation of brown pigment of scalded apples. The investigations of Nagai<sup>6</sup> have shown that the color of aqueous or alcoholic extracts of numerous plant tissues rich in flavones changes to brown or reddish-brown when the extracts are treated with freshly prepared plant juice containing oxidizing enzymes. This color transformation is attributed to the oxidation of certain flavone and flavonol pigments or their glucosides and in accordance with this view Nagai has shown that chemically pure preparations of the pigments themselves yield brownish colors by the action of enzymes. For example, the addition of oxidase to quercetin resulted in the development of a

<sup>6</sup> NAGAI, I. A GENETICO-PHYSIOLOGICAL STUDY ON THE FORMATION OF ANTHOCYANIN AND BROWN PIGMENTS IN PLANTS. Jour. Col. Agr. Imp. Univ. Tokyo 8:1-92, illus. 1921.

deep red color which rapidly changed to brown. Quercitrin, a monorhamnoside of quercetin, under the same conditions yielded a less bright red color than quercetin. It was shown that the brown pigment developed at the expense of flavonol or its glucoside in solution. These results with quercetin or its glucoside and oxidase suggest that the brown pigment appearing in scalded apple peels may be produced by interaction in the tissues of quercetin and oxidase which are normally present in the skin. The observation that brown discoloration of scalded peels is more prevalent on the greener portions lends support to the possibility of quercetin or its glucoside being the parent substance of the brown pigment. The bright-red areas are highly resistant to scald and there is reason for believing that flavonol occurs in smaller quantities in these localities because of its conversion into red or anthocyanic pigment.

It should be borne in mind, however, that other substances in the apple may play a part in the formation under certain conditions of brown or reddish brown color. According to Overholser and Cruess<sup>7</sup> the darkening of cut surfaces of apple tissue may be due to the oxidation of a tannin-like substance. It is interesting to note in this connection that flavonols and tannins are built up of similar nuclei and hence may yield closely related brown products on oxidation.

### SUMMARY AND CONCLUSIONS

(1) One of the first indications of scald in apples is the appearance on the greener portions of the fruit of a typical brown discoloration. A possible relation between the suspected occurrence of a flavonol pigment in the skin and scald led to the isolation and identification of this pigment.

(2) The particular flavonol coloring matter, which was found to occur in McIntosh apple peels, proved to be quercetin,  $C_{15}H_{10}O_7$ . It was identified by means of its penta-acetyl derivative and by combustion of the purified pigment itself.

(3) It is suggested, as a basis for further investigation, that quercetin, or its glucoside which has not been isolated, may be the chromogenic substance which is responsible for the formation of brown pigment in the peels of scalded apples.

<sup>7</sup> OVERHOLSER, E. L., and CRUESS, W. V. A STUDY OF THE DARKENING OF APPLE TISSUE. Calif. Agr. Exp. Sta. Tech. Paper 7: 40 p. 1923.



# GAMETE PRODUCTION IN CERTAIN CROSSES WITH "ROGUES" IN PEAS<sup>1</sup>

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The inheritance of the narrow, pointed stipuled mutation or "rabbit-eared" rogue in garden peas, *Pisum sativum* L., is unusual. Bateson and Pellew<sup>2</sup> found that in crosses with the parent, or broad, obtusely stipuled type, the rogue character is dominant, and that in the F<sub>2</sub> and succeeding generations only rogues appear. There is a failure of the expected segregation into rogue and type plants.

Besides the extremely narrow and the very broad stipuled plants, the same authors<sup>3, 4</sup> described certain intermediate forms which on self-fertilization give varying proportions of rogues, intermediates, and types in their progenies. Intermediates showing a high percentage of rogues among their offspring showed a progressive increase in the percentage of rogues to nonrogues among their progeny when comparing plants derived from the lower nodes with offspring from the upper nodes of the same intermediate plant. The change in ratio between the two classes of plants was accounted for by a corresponding increase among the male gametes of those bearing the rogue factors. The authors point out an association between the excessive production of rogues in the F<sub>1</sub> hybrids and the progressive change in the numerical proportions of rogues to types in the progeny of intermediates on the one hand with a progressive change during growth from type to rogue, in the somatic appearance of both the F<sub>1</sub> hybrids and the intermediates on the other.

In 1923<sup>5</sup> the writer reported the fact of segregation in the F<sub>2</sub> generation of crosses between Gradus rogue and typical plants of Mummy, a nonrogue-producing variety of peas. Although both rogues and nonrogues appeared in the F<sub>2</sub> and F<sub>3</sub> generations of this cross, the ratio between the two classes of segregates did not accord with any known Mendelian system. The rogues greatly outnumbered the nonrogues. The F<sub>1</sub> hybrids resembled in their genetic behavior the intermediates described by Bateson and Pellew, but no data were secured as to the character of the progeny at successive nodes or of the actual ratio of rogue to nonrogue gametes among the ovules and pollen grains.

From the facts obtained, the hypothesis was advanced that rogues arise *de novo* from the parent type by mutation of a single factor, x to X. It is believed that the heterozygous Xx combination is very unstable. In the presence of X

<sup>1</sup> Received for publication April 12, 1924—issued Nov., 1924.

<sup>2</sup> BATESON, W., and PELLEW, C. ON THE GENETICS OF "ROGUES" AMONG CULINARY PEAS (*PISUM SATIVUM*). Jour. Genetics 5:13-36, illus. 1915.

<sup>3</sup> BATESON, W., and PELLEW, C. NOTE ON AN ORDERLY DISSIMILARITY IN INHERITANCE FROM DIFFERENT PARTS OF A PLANT. Proc. Roy. Soc. [London] (B) 89: 174-175. 1916.

<sup>4</sup> ——— THE GENETICS OF "ROGUES" AMONG CULINARY PEAS (*PISUM SATIVUM*). Proc. Roy. Soc. [London] (B) 91: 186-195. 1920.

<sup>5</sup> BROTHERTON, W. E., JR. FURTHER STUDIES OF THE INHERITANCE OF "ROGUE" TYPE IN GARDEN PEAS (*PISUM SATIVUM* L.) Jour. Agr. Research 24: 815-852, illus. 1923.



the factor  $x$  mutates readily to  $X$  during somatic development so that at gametogenesis the  $F_1$  hybrid of type with rogue is homozygous for the rogue factor.

The allelomorphe of  $X$  in Mummy is called  $x'$  and differs from  $x$  of the rogue-producing variety in being more stable, rarely, if ever, mutating to  $X$  when in the homozygous  $x'x'$  condition. However, in the heterozygous  $Xx'$  combination ( $F_1$  and succeeding generations of rogue  $\times$  Mummy) the combination, while more stable than the  $Xx$  germplasm of rogue  $\times$  type, nevertheless is fairly unstable, and  $x'$  mutates frequently to  $X$ . The frequent change of  $x$  or  $x'$  to  $X$  during development of the plant is called mass somatic mutation and the unequal proportion of the two kinds of gametes formed, that is,  $x$  or  $x'$ :  $X$ , results in masking the true Mendelian nature of the inheritance.

The behavior of certain atypical  $F_1$  hybrids of the same cross indicated that occasionally  $x$  and  $x'$  mutate to produce intermediate forms in regard to stipule shape. The inheritance of these latter forms has not been studied.

Since the assumed genetic constitution of the rogue, type and Mummy was derived from the fact of segregation rather than from the actual  $F_2$  ratios observed, it seemed desirable to analyze further the character of the gamete production by the  $F_1$  hybrid of rogue crossed with a nonrogue-producing variety. When out-crossing such an  $F$  hybrid with Gradus type, a rogue gamete uniting with a type gamete would produce a rogue, and a nonrogue gamete uniting with a type gamete would produce a nonrogue plant. This paper is a preliminary report of the results of such an analysis.

The  $F_1$  plants of rogue  $\times$  Mummy are very late in flowering and therefore difficult to cross with the early-flowering Gradus type. For this reason the  $F_1$  plants of rogue  $\times$  Rice's 330 (a nonrogue-producing variety) were substituted. Rice's 330 is an early-maturing sort with stipules intermediate in size and shape between Gradus type and Gradus rogue. The mean ratio of  $\frac{\text{length of stipule}}{\text{width of stipule}}$  as determined by measurements of four stipules from the upper part of mature plants for the three parental types was as follows: Gradus type,  $1.693 \pm 0.0107$ ; Gradus rogue,  $2.339 \pm 0.0096$ ; and Rice's 330,  $1.991 \pm 0.0086$  (Table I).

In 1919; 36  $F_1$  hybrids of Gradus type  $\times$  Rice's 330 were grown and found to have a mean stipule ratio of  $1.825 \pm 0.0098$ , i. e., intermediate between the two parents (Table I). The stipules were larger than those of Rice's 330 and narrower than Gradus type, but had the emarginate apices typical of Gradus rather than the more acutely pointed stipules of Rice's 330.

During the summer of 1920, the writer was unable to have stipule measurements taken until very late in the season, at which time the foliage of only a few of the late flowering segregates was still green enough to measure. In all 87 plants from 21 families were measured. The mean, standard deviation, and coefficient of variation are given in Table I.

The  $F_2$  segregates were not classified by inspection, and since the data are meager, no attempt is made to analyze the factorial differences between the two parents. The data are given at this time merely for comparison with that which follows.

The  $F_2$  hybrids of Rice's 330  $\times$  Gradus rogue and reciprocal, 32 in all, grown in 1919, were intermediate between Rice's 330 and Gradus rogue in appearance, with a mean stipule ratio of  $2.185 \pm 0.0213$  (Table I).

The  $F_2$  generation consisted of 47 families, from which the mean stipule ratios of 247 plants were obtained. The range of variation includes that of both parents and indicates segregation into rogue and nonrogue forms (Table I). No data are available as to the exact segregation, since the plants were not classified by stipule shape.

TABLE I.—Frequency distributions and statistical constants for ratio of length of stipule for *Gradus* type, *Gradus* rogue, *Rice's 330*, and various  $F_1$  and  $F_2$  hybrids, and back-cross segregates

	1.35	1.45	1.55	1.65	1.75	1.85	1.95	2.05	2.15	2.25	2.35	2.45	2.55	2.65	2.75	2.85	2.95	Total	M.	$\sigma$	C. V.
<i>Gradus</i> type ( $P_1$ )	1	0	16	64	33	18	3	7	21	33	30	22	10	8	4	—	—	135	1.693+0.0107	0.098+0.0075	5.78+0.2381
<i>Gradus</i> rogue ( $P_1$ )	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	135	2.339+0.0096	.165+0.0068	7.06+0.2912
<i>Rice's 330</i> ( $P_1$ )	—	—	—	—	3	9	17	27	7	—	—	—	—	—	—	—	—	63	1.991+0.0086	.101+0.0060	5.07+0.3504
$F_1$ ( <i>Gradus</i> type $\times$ <i>Rice's 330</i> )	—	—	—	1	14	15	6	1	—	—	—	—	—	—	—	—	—	36	1.825+0.0098	.088+0.0069	4.82+0.3832
$F_2$ ( <i>Gradus</i> type $\times$ <i>Rice's 330</i> )	—	—	1	8	14	22	21	12	5	4	—	—	—	—	—	—	—	87	1.899+0.0113	.157+0.0080	10.44+0.4931
$F_1$ ( <i>Gradus</i> rogue $\times$ <i>Rice's 330</i> )	—	—	—	—	—	3	1	6	6	7	6	3	—	—	—	—	—	32	2.185+0.0213	.179+0.0151	8.19+0.6950
$F_2$ ( <i>Gradus</i> rogue $\times$ <i>Rice's 330</i> )	—	—	—	—	—	11	16	26	21	28	48	50	30	24	13	8	1	278	2.350+0.0101	.081+0.0046	4.63+0.2664
$F_1$ ( <i>Gradus</i> rogue $\times$ <i>Rice's 330</i> ) $\times$ type, all segregates	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	138	1.975+0.0159	.278+0.0112	14.07+0.5794
$F_1$ ( <i>Gradus</i> rogue $\times$ <i>Rice's 330</i> ) $\times$ type, nonrogue segregates	—	3	13	38	14	10	12	13	11	12	7	2	1	2	—	—	—	69	1.748+0.0065	.081+0.0046	4.63+0.2664
$F_1$ ( <i>Gradus</i> rogue $\times$ <i>Rice's 330</i> ) $\times$ type, rogue segregates	—	—	3	13	13	2	—	—	—	—	—	—	—	—	—	—	—	69	2.223+0.0155	.192+0.0110	8.63+0.4622

However, some idea of the character of the  $F_2$  segregation following the cross between rogue and Rice's 330 may be obtained from a classification of 262  $F_2$  plants grown in 1923 from 5  $F_1$  hybrids grown in 1922 under a wire cage to protect them from volunteer crossing by insects. The  $F_2$  plants were grouped as rogues, intermediates with pointed stipules of greater width than in typical rogues, and as broads with large stipules resembling Rice's 330 or Gradus type. The variability of the rogues and intermediates was such that it was not always easy to separate them, but the differences between the two roguelike classes and the broads or type-like plants were easily recognized. In all there were 217 rogues, 37 intermediates, and 8 broads, clearly not a typical Mendelian segregation.

During the winter of 1922-23 there were grown 155 plants from 32 back-crosses of the kind [(rogue  $\times$  Rice's 330)  $\times$  type], as well as 8 of the reciprocal back-crosses. The  $F_1$  hybrids used as either pollen or pistillate parents were the same 5 plants whose  $F_2$  progeny, derived from self-fertilized seed, were grown in 1923 and described above.

The progeny of these back-crosses were for the most part easily distinguished as broads with wide, rounded stipules, or as rogues with characteristic narrow, pointed stipules. The stipule differences between these two classes of segregates is clearly shown by the data for their mean stipule ratios given in Table I. The frequency distribution and mean of  $1.748 \pm 0.0065$  for the broad segregates are very near those of the  $F_1$  hybrids of Rice's 330  $\times$  Gradus type. The variability in stipule shape among the broad segregates, as shown by the coefficient of variation, is also strikingly near that of the  $F_1$  generation of the latter, i. e.  $4.63 + 0.2664$  and  $5.07 + 0.3054$ , respectively.

The narrow or roguelike segregates resemble closely the pure rogues in regard to their frequency distribution, except that the range of variation of the hybrids extends below 2.05, the lowest class of pure rogues. The mean,  $2.223 \pm 0.0155$ , is significantly different from that of  $2.339 \pm 0.0096$  for the pure rogues, but is also decidedly different from that of the broad (nonrogue) segregates.

The roguelike plants also resemble the rogue parent in the great variability they exhibit, caused in part by the presence of chimera-like segregates characterized at the middle and upper nodes by having the corresponding stipules of each pair unlike, that is, one stipule narrow and pointed while the other stipule would be broad and obtuse. In all, 13 chimeras were identified, 10 from seed pollinated by the type and 3 from the reciprocal cross. The following measurements of the stipules at successive nodes are typical of this class. The ratios for the pair of stipules from the same node are inclosed in brackets and the largest ratio, usually above 2.05, indicates in every instance the roguelike stipule.

Plant No. ....	3. 100-2	3. 111-2	3. 118-1
	(1. 71)	(1. 66)	(1. 86)
	(2. 76)	(1. 81)	(2. 55)
	(1. 87)	(1. 63)	(1. 55)
	(3. 36)	(2. 33)	(2. 77)
	(1. 53)	(1. 82)	(1. 92)
	(2. 09)	(2. 36)	(2. 66)
Average .....	2. 05	1. 93	2. 21

In the data given in Table I the chimeras are classed as rogues, presumably heterozygous for the rogue factor, as shown by the presence of both the pointed and obtuse stipules. Whether the chimeras are merely heterozygous forms or are mosaics in the sense of Bateson and Pellew is not known, since each plant produced only two or three seeds in the greenhouse, and these too late for spring planting.

It is hoped to have data soon on the genetic behavior of comparable material from similar back-crosses made during the past summer.

The classification of the  $F_2$  segregates according to their nodal origin from the  $F_1$  hybrid is shown in Table II. There is a slight tendency for the proportion of rogues to nonrogues (broad) to increase toward the upper nodes, but as the total number is small, emphasis in the interpretation can not be put on this feature of the data. There may be an excess of nonrogue gametes produced by the ovules and an excess of rogue gametes produced by the pollen grains. However, the numerical differences between the two classes are slight enough to be well within the experimental error for so few plants if the real gametic ratio between the rogues and nonrogues was 1:1, irrespective of which way the cross was made. The ratio of rogues to nonrogues for both crosses combined actually is 77:78, or practically 1:1.

On the other hand, the occurrence of a large number of rogues in the  $F_2$  generation above the proportion theoretically expected for a monohybrid ratio can be explained by assuming the probable excess production of rogue-bearing male gametes indicated by the  $F_1$  back-crosses actually occurred, especially at the upper nodes. The five  $F_1$  plants used were exceptionally tall and flowered over a much longer period than the Gradus types. Consequently the back-crosses were made with flowers largely from the eighth to the fourteenth nodes of the hybrids. The self-fertilized plants came for the most part from the fourteenth to the twentieth nodes on the main stem, or from branches of the main stem produced late in the season.

From the foregoing it appears that the zygote heterozygous for the rogue factor is unstable. Apparently the presence of the rogue factor causes a change or mutation of the allelomorphic factor which entered the hybrid from Rice's 330. This genetic change during the development of the plant is most often due to the rogue factor itself, but occasionally it may take some other form, or the rogue factor itself may change. In either event a roguelike plant, possibly the  $F_2$  intermediates or the chimeras here described, is the outward expression of the change.

A comparable case has been reported by Blaringhem<sup>6</sup>, who studied the behavior of a mutation in *Pisum* with green cotyledons from a variety with yellow cotyledons. On self-fertilization the green-seeded peas gave varying proportions of plants with green, yellow, and variegated green and yellow seeds. The yellow seeds breed true, while the variegated and green seeds repeat the splitting. Seven self-fertilized green-seeded plants gave only green-seeded plants from the upper and lower nodes, while the middle nodes gave all three types. The totals for all seven plants were 118 green, 26 variegated, and 17 yellow. This author describes the phenomenon as one of hereditary mosaic, evidently believing that the green plants are a genetic mosaic of green and yellow and that "disjunction" of the two characters takes place under certain environmental conditions. He suggests that the manifestation of the true nature of the green-seeded plant or mosaic is dependent upon the age of the plant and the environment, especially temperature and humidity.

As Bateson and Pellew point out in discussing the genetic behavior of the intermediates they have described, where the gametic output is so fluctuating it is perhaps unwise to hypothecate a factorial system to interpret the results. Nevertheless, it is believed that here the presence of rogue and non-rogue gametes in the ratio of approximately 1:1, at the lower nodes at least of the  $F_1$  hybrid between Rice's 330 and rogue, indicates a single-factor difference

<sup>6</sup> BLARINGHEM, L.—HÉRÉDITÉ ANORMALE DE LA COULEUR DES EMBRYONS D'UNE VARIÉTÉ DE POIS (*PISUM SATIVUM* L.). *Compt. Rend. Acad. Sci. [Paris]* 174:877-879. 1922.

between the two in regard to the rogue character. It would appear that subsequently the original 1:1 gametic ratio is disturbed by somatic mutation, so that from the upper nodes of the main stem and from its branches an excess of rogue gametes is produced. No attempt has been made to determine what external influences, if any, affect the gametic ratio.

TABLE II.—Distribution according to nodal origin from the *F*<sub>1</sub> hybrid between Rice's 330 and rogue of self-fertilized progeny and of progeny derived from back-crosses with *Gradus* type

	Hybrid ♀			Hybrid ♂			Self-fertilized			
	Non-rogue	Rogue	Ratio	Non-rogue	Rogue	Ratio	Non-rogue	Inter-mediate	Rogue	Ratio
8.....	4				2					
9.....	2	3	1:1.5	9	9	1:1.0			8	
10.....	3	3	1.0	2	9	4.5	2	1	15	1:8.0
11.....	12	5	0.4							
12.....	7	3	0.4				1		3	3.0
13.....	4	3	0.7					2	9	
14.....	9	8	0.9	2	4	2.0	1	2	8	10.0
15.....	6	9	1.5				1	3	10	13.0
16.....	4	7	1.7					6	7	
17.....	2	2	1.0				1	2	27	29.0
18.....		2						10	16	
19.....								1	15	
20.....								2	17	
5.....									4	
6.....								1	3	
7.....								2	3	
8.....								2	2	
9.....										
10.....	4	4	1.0						5	
12.....	2						2	1	4	2.5
13.....	1	3	3.0						5	
14.....									18	
15.....	4	2	0.5						11	
16.....								1	18	
17.....								1	5	
18.....									4	
Total.....	64	54	1:0.8	13	24	1:1.8	8	37	217	1:31
								254		

# SURVEY OF BLISTER RUST INFECTION ON PINES AT KITTERY POINT, MAINE, AND THE EFFECT OF RIBES ERADICATION IN CONTROLLING THE DISEASE<sup>1</sup>

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## PINE INFECTION SURVEY, 1916-1918

In 1916 an outbreak of white pine blister rust (*Cronartium ribicola*, Fischer) was discovered in the southern part of York County, Maine. Extensive scouting during the same season revealed the rust present in varying degrees of abundance throughout southern Maine and adjacent New Hampshire. The oldest and severest attacks of the disease found in these regions were upon stands of native white pine (*Pinus strobus*, L.) near a patch of cultivated black currants (*Ribes nigrum*, L.) at Kittery Point, Me. These currants had been brought from Nottingham, England, during the summer of 1897 and planted in a small garden surrounded by nearly pure stands of second growth white pine comprising mixed age classes. Systematic inquiry and search failed to show that any foreign white pine stock had been planted at or near Kittery Point, thus contradicting the supposition that the disease became established through that medium. The maximum ages of the more advanced rust lesions on pines in the immediate vicinity indicate that the establishment of blister rust at Kittery Point was coincident with the time when the patch of imported black currants was planted. This is further evidenced by the fact that the oldest pine infections found in that region are in the immediate vicinity of these currant bushes.

In 1917 approximately 6 square miles of the infested area at Kittery Point were included in an experimental local control tract on which both wild and cultivated currant and gooseberry bushes were located and uprooted by systematic crew work.<sup>2</sup> Before eradication began a 31.5-acre block surrounding the patch of imported black currants was plotted and each pine, currant, and gooseberry located for detailed investigation.

In 1918 careful studies were made of conditions on the 31.5-acre tract to determine the severity of infection on pines and other facts relative to the spread of the rust. Results of these investigations are shown in the charts and tables which accompany the following discussions.

## DISTRIBUTION OF PINE INFECTIONS WITH RELATION TO THE PRESENCE OF RIBES AND THE INFLUENCE OF STORM WINDS

The effect of different patches of *Ribes* in causing blister rust infection on pines was evidenced by the condition of the trees and stands nearest these bushes. In cases where the *Ribes* had caused pine infection, the amount of infection on pines varied according to the species, size, and exposure of the *Ribes* and was greatest on near-by pines. In several instances the *Ribes* were small and densely screened and apparently had not caused pine infection. (See fig. 1.)

<sup>1</sup> Received for publication May 9, 1924—issued Nov. 1924.

<sup>2</sup> A crew consisted of 4 to 6 men in line, followed by a foreman. Intervals between line men varied according to the density of ground cover. In dense brush the interval was 6 feet or less while in open tracts the interval increased up to 20 feet. Efficiency was maintained by the constant checking of the foreman and by the crew systematically reworking definite units.

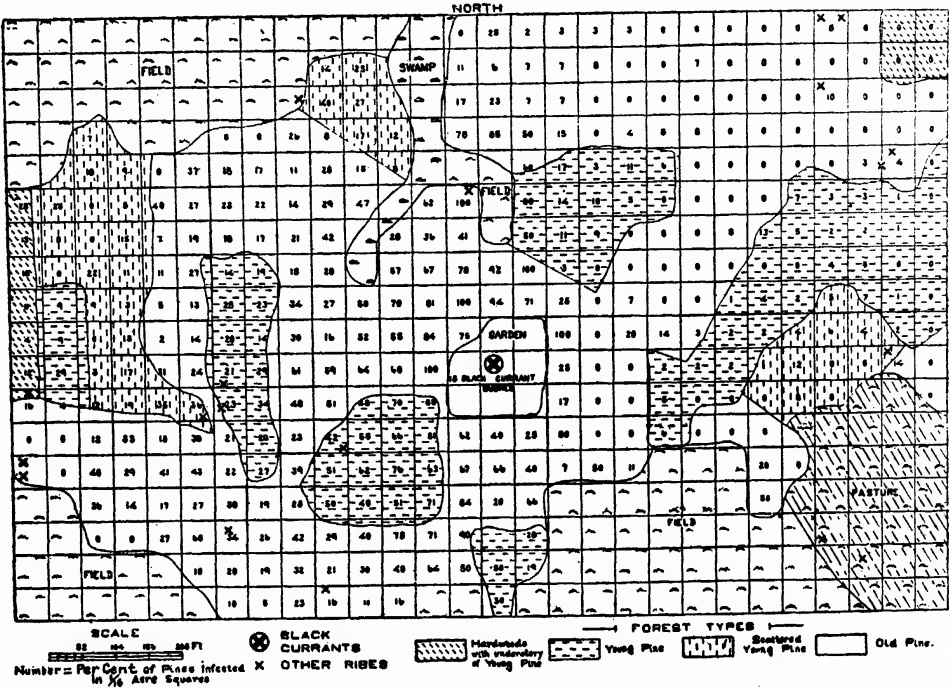


FIG. 1.—Compilation of data collected on the 31.5-acre experimental area in 1918. Each square represents one-sixteenth acre, and the numbers in the squares represent the percentage of pines infected when the pines were examined in detail in 1918.

INFLUENCE OF WIND

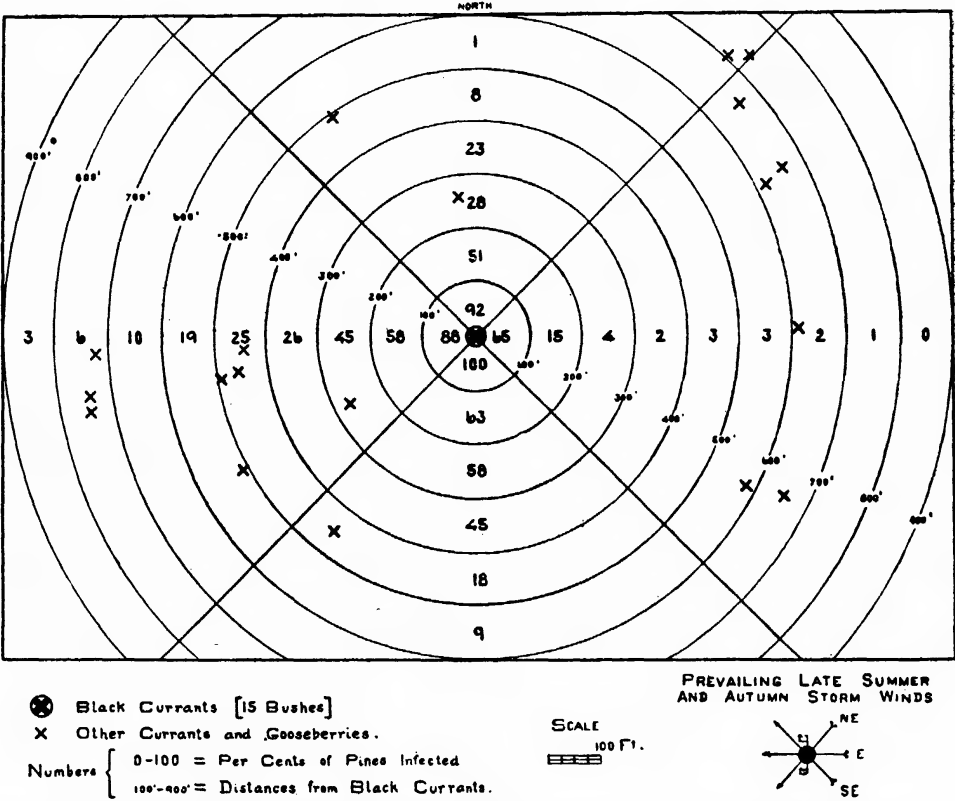


FIG. 2.—Data collected on the 31.5-acre experimental area. Figures represent per cent of infected pines in each quadrant by 100-foot zones.

The predominant effect of the patch of cultivated black currants in causing infection as compared to the possible effect of the other Ribes found on the area is shown in figure 2. The percentage of infected trees in concentric 100-foot zones from the black currants shows pronounced and fairly regular decreases in the four directions of the compass, whereas this would not be the case in similarly spaced zones circumscribing any other point as an infection center. The highest percentages of infection, when considered in relation to the location of the black currants, are in the paths of the late summer and autumn storm winds. On this basis, infection emanating from the patch of black currants was traceable in diminishing degrees for distances of 600 to 900 feet while infection from other Ribes was comparatively meager on nearby pines and not traceable beyond 200 feet.

RATE OF INFECTION OF STAND

The blister rust became measurably established on pines in the west half of the area studied at Kittery Point in 1901. From 1901 to 1916, inclusive, 34.5 per cent of the trees became infected. The rate at which the stand became infected increased from a yearly average of 0.225 per cent during the first 4-year period to a yearly average of 4.6 per cent during the last 4-year period preceding the eradication of Ribes.

TABLE I.—Rate of infection of stand <sup>a</sup>

	First 4-year period (1901–1904)	Second 4-year period (1905–1908)	Third 4-year period (1909–1912)	Fourth 4-year period (1913–1916)
Total number of healthy trees at beginning of 4-year period.....	6, 727	6, 666	6, 317	5, 386
Number healthy trees that became infected during the 4-year period.....	61	349	931	986
Per cent of healthy trees that became infected during 4-year period.....	0. 9	5. 2	14. 7	18. 3

<sup>a</sup> Table I is compiled from the data on the west half of the experimental area shown in Figure 1.

SEVERITY OF INFECTION ON PINES OF DIFFERENT SIZE CLASSES

The percentage of infection was lowest among trees of the smallest size and highest among trees of the largest size. Table II indicates that on the area studied the percentages of trees infected are correlative to their diameter-size classes.

TABLE II.—Percentage of pines infected, by diameter classes <sup>a</sup>

Diameter breast high	Number of pines	Trees infected
<i>Inches</i>		<i>Per cent</i>
1 and under.....	3, 752	21
2.....	700	34
3.....	412	43
4.....	338	43
5.....	264	42
6.....	165	61
7.....	160	54
8.....	169	62
9.....	155	59
10.....	149	71
11.....	130	60
12.....	109	66
13 and over.....	224	79

<sup>a</sup> Table II is compiled from the data on the west half of the experimental area shown in Figure 1.



## SEVERITY OF INFECTION IN PINE STANDS OF DIFFERENT DEGREES OF STOCKING

In different units of the area, similarly exposed to *Ribes*, the percentages of infected trees varied according to the degrees of pine stocking. The percentages of infected trees were highest in understocked stands and lowest in overstocked stands. Figure 3 shows the percentages of trees infected in stands of the area having different degrees of pine stocking.

### PINE INFECTION SURVEY, 1921

In 1921 an inspection was made of the entire experimental control tract at Kittery Point, where the *Ribes* eradication work had been done in 1917, to ascertain the effectiveness of this work in preventing further infection of the pine. No pine infection occurring since 1916 was found except in isolated portions of

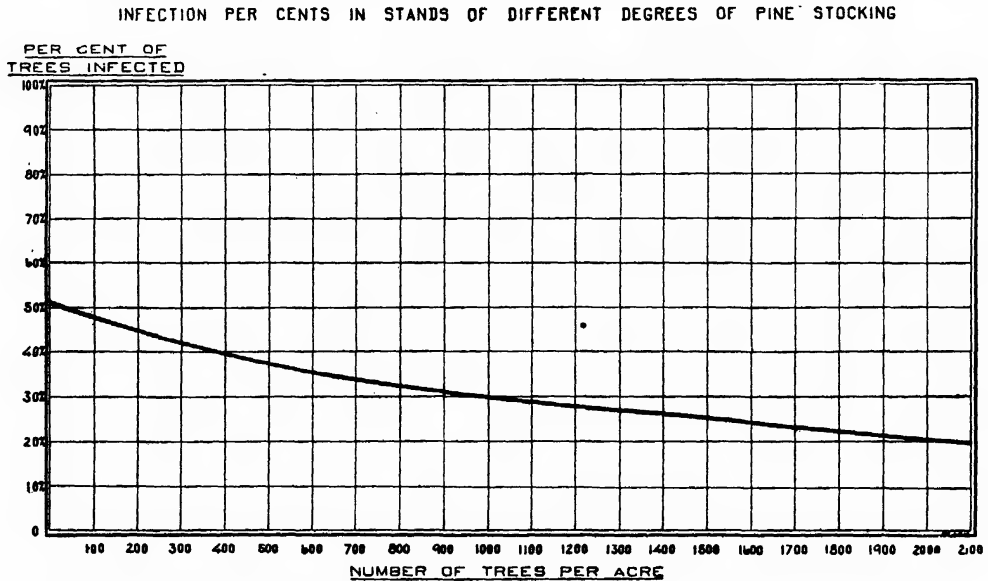


FIG. 3.—Compiled from the data on the west half of the experimental area shown in fig. 1

the tract where an occasional *Ribes* bush had been missed by the eradication crews or new ones had grown since 1917. Detailed investigations were made on thirty-two  $\frac{1}{8}$ -acre plots located in the experimental area where all of the *Ribes* bushes had been destroyed in 1917 and no new bushes had grown since that time, to determine the condition of the pines and the changes which had taken place in the stands.

On the 32 plots there remained 1,737 trees of the original stand studied in 1918. Of these, 31 per cent or 546 trees had been killed by the blister rust as a result of infections which occurred prior to the eradication of *Ribes*. Painsstaking examination of the remaining 1,191 live trees of the original stand and 485 young trees that had seeded in on the area since 1917 showed that no new infections had occurred since 1916.

There was a marked variation in the severity of infection of different crown classes (fig. 4.) The dominant trees, which are of greatest economic importance in the stand, were subject to the highest degree of infection from blister rust.

## SEVERITY OF BLISTER RUST INFECTION IN DIFFERENT CROWN CLASSES

## PERCENTAGE OF PINES INFECTED BY CROWN CLASSES

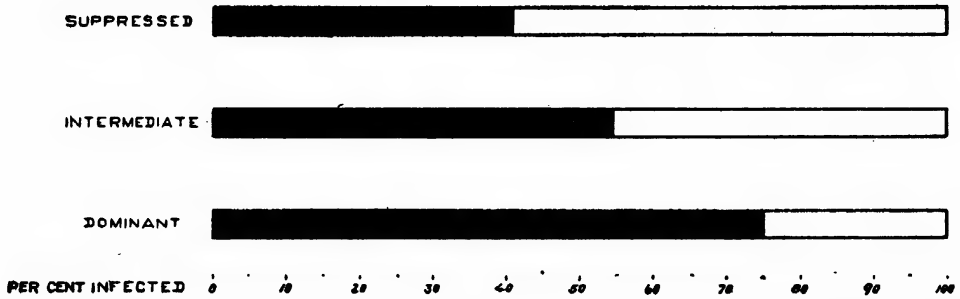


FIG. 4.—A compilation of data for all trees on thirty-two  $\frac{1}{8}$ -acre plots located 100 to 400 feet from the site of the imported black currants in the west half of the area shown in fig. 1

## CONDITION OF THE STAND

As shown in figure 5, there has been a constant increase in the number of trees dying from the effect of blister rust, while the number of infected trees has remained the same as in 1917. The number of healthy trees has been augmented by 485 young pines which seeded in during the four-year control period. This demonstrates that the destruction of *Ribes* prevented the occurrence of new infections and allowed pine reproduction to continue at an average annual rate of 60 trees per acre.

## CONDITION OF STAND ON CONTROL AREA IN 1917 AND 1921

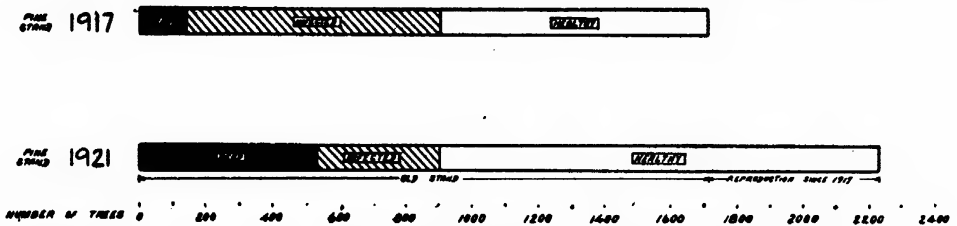


FIG. 5.—A compilation of data collected on the thirty-two  $\frac{1}{8}$ -acre plots described under fig. 4

## SUMMARY

Investigations reported in this paper indicate that a patch of cultivated black currant plants introduced from England in 1897 and planted at Kittery Point, Me., during the same year, established a primary infection center of the white-pine blister rust whence the disease became widely spread through the neighboring white-pine forests. On the basis of detailed investigations conducted on experimental areas selected in and about the primary infection center, the following facts are evidenced:

1. Cultivated black currants were the *Ribes* mainly responsible for causing the pine infection on the area studied.
2. In cases where either wild or cultivated *Ribes* bushes had caused pine infection, the greatest amount of infection occurred in the nearest pine stands, and the infection from these bushes rapidly decreased at greater distances.
3. The highest degrees of pine infection were in the paths of late summer and autumn storm winds.

4. The average annual rate at which the pine stands became infected constantly increased over a period of 16 years and in 1916 the rate of infection was 20 times the rate in 1901.

5. The infection percentages in pine stands varied according to the diameter classes of the trees, being highest among the larger size classes.

6. The infection percentages in pine stands varied according to the degree of pine stocking, being highest in understocked stands.

7. The infection percentages in pine stands varied according to crown classes, being highest among dominant trees.

8. Destruction of all *Ribes* prevented the occurrence of new pine infections and permitted the area to be naturally restocked with disease-free seedlings.

# INDEX

	Page		Page
Abortion—		Anthelmintics, literature.....	337
bovine, infections, immunology, studies.		Antimony, insecticidal value.....	395, 396
By J. M. Buck and G. T. Creech.....	607-642	<i>Apelencus ritzemabosi</i> , description and oc-	
infections—		currence on chrysanthemums.....	1061-1062
infection through injection of infected		Apple—	
material.....	620, 621	blackrot—	
of cattle, immunology, studies and experi-		fungus.....	583-488
ments.....	607-642	review of work.....	589-590
Acala cotton—		maggot, anatomy and metamorphosis.....	1-36
growing and characters, comparisons.....	938-952, 954	peels, quercetin isolation and identifica-	
spacing experiments in Southern Cali-		tion.....	1243-1245
fornia.....	1081-1093	Arizona—	
Acid, production by <i>Bacillus larvae</i> .....	153-155	cottons—	
Acromania, cotton disease.....	803-828	comparison.....	938-954
Aecidiospores—		leaf-tissue fluids, chlorid content deter-	
expulsion by the mayapple rust.....	923-926	mination.....	695-704
uninucleated, in <i>Caeoma nitens</i> .....	1045-1058	Gila Valley, physiography, soils, and	
Agglutination, tests for infectious abortion		vegetation.....	724-725, 728-730
in cattle.....	613-614, 617-620	Arkansas, strawberry leather rot.....	368-371
Alabama, climate, comparison with Japan.....	244-253	Arlington Experiment Farm, wheat-rye	
Alaska spring wheat, crosses with <i>vulgare</i>		hybrids.....	567-568, 573-575
species, studies of results.....	1018-1031	Arrowweed, association with saltbush in	
Aldous, A. E., and Shantz, H. L.: Types of		Southwestern desert soils.....	770-772, 795-799, 800
Vegetation in the Semiarid Portion of the		Ascarids, control in foxes.....	334-337
United States.....	99-123	Ascaris—	
Alfalfa, wild, indicator of land value.....	101,	horse, toxicity.....	577-582
	103, 117, 119	<i>lumbricoides</i> , toxicity.....	577-582
Alfalfa, susceptibility to curly-top.....	314	sensitization.....	577-582
Alimentary canal, bee larva.....	1184-1188, 1206	Ascaron, active principle of Ascaris.....	579
Alkali salts, Tempe, Ariz., chemical analy-		Ascigerous stage of <i>Fusarium moniliforme</i> ,	
ses.....	726	and synonymy.....	909-922
Alkaloids, insecticidal value.....	400	Ash—	
Allen, Ruth F., and Mackie, William W.: The		constituents in meats.....	340
Resistance of Oat Varieties to Stem		lumber, steaming for control of powder-	
Rust.....	705-720	post beetle.....	1033-1038
Allison, F. E.—		Aspen, indicator of land value.....	116, 118, 123-125
Chemical and Biological Studies with		Atriplex—	
Cyanamid and Some of Its Transforma-		<i>fasciculata</i> , on southwestern desert soils.....	778
tion Products.....	37-69	<i>lentiformis</i> , indicator of land value.....	113, 122
Greenhouse Experiments with Atmos-		<i>Avena</i> spp., inoculation with stem rust, tests	
pheric Nitrogen Fertilizers and Related		of resistance.....	706-709
Compounds.....	971-976	Bacilli, causes of fowlbrood.....	129-168
The Effect of Cyanamid and Related Com-		<i>Bacillus</i> —	
pounds on the Number of Microorgan-		<i>alvei</i> , discovery and relation to fowlbrood.....	131-133
isms.....	1159-1166	aromatic, new species from dairy wastes.....	275-276
The Nitrification of Phosphorus Nitride..	1117-	<i>brandenburgiensis</i> , same as <i>Bacillus larvae</i> .....	132, 133
	1118	<i>larvae</i> —	
Alsberg, Carl L., and Schwartze, Erich W.: Pharmacology of Gossypol.....	191-197	biochemical reactions, sup-	
Relation Between Toxicity of Cottonseed		plemental studies.....	150-157
and its Gossypol Content.....	173-189	growth in cultures, relation to sugar con-	
Amby wheat, crosses with emmer species,		centration.....	135-141
studies.....	1019-1031	Back, E. A., and Cotton R. T.—	
Ammonia—		Effect of Fumigation Upon Heating of Grain	
determination in nitrogenous materials..	527-539	Caused by Insects.....	1103-1116
nitrogen, determination method.....	41-42	Effective Use of Hydrocyanic-acid Gas in	
Ammonium sulphate—		the Protection of Chick-peas Warehoused	
effect on numbers of bacteria in soil.....	1160, 1161	in 240-Pound Sacks.....	649-660
nitrification rates, comparisons.....	43,	Relative Resistance of the Rice Weevil and	
	54-56, 58-60, 65	the Granary Weevil to High and Low	
Analysis—		Temperatures.....	1043-1044
methods for meats.....	339-341	Bacteria—	
rye culms, normal and brittle.....	170	cottonseed samples, effect on germination.....	286
Anatomy, apple maggot.....	1-36	soil, numbers, effect of cyanamid and re-	
<i>Anchusa</i> spp., hosts of <i>Puccinia dispersa</i> ,		lated compounds.....	1159-1166
and infection methods.....	1119-1124	Bacterial leafspot of Delphinium.....	261-270
<i>Ancylostoma caninum</i> , occurrence in foxes....	331	<i>Bacterium</i> —	
Anderson, M. S.: The Heat of Wetting of Soil		<i>abortus</i> cultures, use as vaccine to prevent	
Colloids.....	927-935	abortion.....	609-620
Angoumois grain moth, wheat infestation....	1107,	<i>delphinii</i> , morphology, cultural characters,	
	1108, 1115	and description.....	264-269
Animals—		<i>flaccumfaciens</i> , cause of bean wilt.....	490
immunization experiments for bovine abor-		<i>phaseoli</i> , cause of bean blight.....	490
tion, detailed records.....	621-642	<i>sanguinarium</i> , morphology.....	75-76
meat, edible viscera.....	339-346	<i>translucens</i> , cause of bacterial blight of rye.....	1039
undergoing gossypol poisoning, metabo-		Barberry, infection with <i>Puccinia graminis</i>	
lism.....	192-194	<i>poae</i> , and spread of rust.....	541-542, 547
Antelope brush, indicator of land value.....	109,	Barium—	
	118, 122-126	carbonate, insecticidal value.....	395, 396
Anthelmintic experiments with foxes.....	331-337	oxalate, insecticidal value.....	395-396

	Page		Page
Barley, leaf rust, <i>Puccinia anomala</i> , aecial stages.....	1119-1126	Bones, skull, meadow mouse, description and variation.....	1001-1009, 1014
Baston, G. H.: Studies of Wheat in Storage, and Heat Caused by Insects.....	1103-1104	Borax, use in treatment of citrus fruits for the prevention of blue mold rot.....	961-968
Bean—		Bordeaux spray, use against pecan scab.....	326-327, 329
disease, seed-borne, effect of moisture.....	489-497	<i>Botryosphaeria ribis</i> —	
foliage burning, insecticides testing.....	402	association with <i>Physalospora malorum</i> on currant.....	584
weevil, four-spotted, control, in chick-peas by hydrocyanic-acid gas.....	649-660	<i>chromogena</i> on apple and currant.....	594-596
weevils, fumigation.....	347-356	comparison with <i>Physalospora malorum</i> .....	596-597
Beans—		life history.....	590
fertilizer experiments.....	972-974	on currant and apple.....	589-598
heating by insects, notes.....	1105, 1114	Botrytis brown rot of strawberries, cause and description.....	646, 647, 648
navy, growing and yields, in seed-soaking experiments.....	490-496	Bots, ox, observations.....	271-274
Bee—		Bowman, John J., and Fulton, Harry R.: Preliminary Results with the Borax Treatment of Citrus Fruits for the Prevention of Blue Mold Rot.....	961-968
diseases, literature cited.....	165-168	Brachysm, cotton growth disorder.....	806-807
larva, morphology.....	1167-1208	Braham, J. M.: Chemical and Biological Studies with Cyanamid and Some of its Transformation Products.....	37-69
larvae, effects of bacterial metabolism.....	163-164, 165	Brain, bee larva, description.....	1172-1176, 1205
Bees—		Brains, composition.....	344
brood—		Bran, heating by <i>Silvanus cassiae</i> .....	1105
diseases, relation to life history of bee larvae.....	130-135, 159-164	Branching, abnormal, of cotton, effects of crazy-top and other diseases.....	813-814
food, composition.....	141-142	Braun, Harry: A Gradient of Permeability to Iodin in Wheat Seed Coats.....	225-226
foulbrood, causes and development.....	130-135	Breeding—	
larvae—		chicken experimental study of sex ratio.....	200-220
composition at different ages.....	142-144	corn for improvement.....	435-436
feeding, cocoon-spinning, and quiescent stages.....	159-163	Brewbaker, H. E.: Brittle Straw and Other Abnormalities in Rye.....	169-172
life history relation to brood diseases.....	130-135, 159-164	Briques, colloids, making, drying, and testing.....	501-511
race, sex, and age, relation to foulbrood.....	130-132	Brittle straw, rye, and other abnormalities.....	169-172
Beetle—		Brood diseases, bee, relation to life history of bee larvae.....	130-135, 159-164
lady, squash, insecticides.....	401	Brooks, Fred E., and Cotton, R. T.: The Cambium Curculio <i>Conotrachelus anaglypticus</i> .....	377-386
powder post, temperatures fatal, determination.....	1033-1038	Brotherton, Wilber, Jr.: Gamete Production in Certain Crosses with "Rogues" in Peas.....	1247-1252
Beetles, Japanese, insecticides.....	396-402	Brown rot of fruits in America, fungus causing.....	955-960
Beets—		Bruchids, control in chick-peas by fumigation.....	649-660
age of plants, relation to curly-top susceptibility.....	313-314	<i>Bruchus</i> —	
growth and weight, effect of curly-top.....	303-304	<i>obtectus</i> , fumigation methods and results.....	347-356
inoculation to produce curly-top.....	304-306	<i>quadrinaculatus</i> —	
losses from curly-top in California, 1917-1920.....	301	control in chick-peas by hydrocyanic acid gas.....	649-660
planting early to avoid leafhoppers.....	316	fumigation, methods and results.....	347-356
resistant strains to curly-top disease.....	317	<i>See</i> Bean weevil, four-spotted.	
sugar, curly-top disease, studies.....	297-320	<i>Bruchus</i> spp., heat of grain, notes.....	1105, 1106, 1109-1112, 1114-1115
Bibliography—		Brush varieties, indicator of land value.....	101, 108, 109, 114, 115, 117-127
bee diseases.....	165-168	Bryan, Mary K.: Bacterial Leafspot of Delphinium.....	261-270
cereal rusts.....	718-719	Buck, J. M., and Creech, G. T.: Studies Relating to the Immunology of Bovine Infectious Abortion.....	607-642
corn ear molds.....	921-922	Buckwheat, shrub, indicator of land value.....	112, 118, 121
cotton growth diseases.....	897	Bud—	
curly-top of beets.....	318-319	abortion in Pima cotton, non-inheritance.....	2041-1042
cyanamid.....	68-69	selection, relation to quality of crop in the Washington navel orange.....	521-526
honeybee larva.....	1208-1212	variations, citrus, progeny tests and performance records.....	522-525
insect anatomy and metamorphosis.....	33-36	Buds, citrus, progeny propagation and performance records.....	523-525
plant growth factors.....	900-907	Buffalo grass, indicator of land value.....	102, 103, 117-121, 124
sex studies of fowls.....	221-224	Bulb rot, <i>Fusarium</i> , of onion and relation to environment.....	683-694
sweet corn.....	440-443	Cabbage—	
Biguanid—		clubroot infection, relation to temperature and moisture.....	553-557
nitrate, effect on numbers of bacteria in soil.....	1165, 1166	"hillup" to control clubroot.....	557-558
nitrification in soils.....	40, 65	Cacti, indicators of land value.....	112, 118, 121, 122, 127
Binding power, soil colloids, factors influencing.....	499-513		
Black spot, delphinium. <i>See</i> Leafspot, bacterial, of Delphinium.			
Blackberry, orange rust, uninucleated acidiospores.....	1045-1058		
Blackrot, apple—			
fungus, comparison with fungus of currant cane blight.....	596-597		
fungus on apple and currant.....	583-588		
review of work.....	589-590		
Blemishes, orange, relation to quality.....	521		
Blight—			
bacterial, of rye.....	1039-1040		
bean, cause and comparison with wilt.....	490		
Blister rust infection, survey, on pines at Kittery Point, Me.....	1253-1258		
Blossoming, effect of varying length of day.....	447-453		
Blue mold rot, citrus fruits, prevention, borax treatment.....	961-968		
Bolls, cotton—			
dwarfing, effects of crazy-top and other diseases.....	818-819		
shedding and development, comparisons.....	943-952		

	Page		Page
<i>Caecoma nitens</i> —		Chickens—Continued.	
occurrence without spermatogonia.....	1047-1048	sex ratio—	
uninucleated aecidiospores and associated phenomena.....	1045-1058	experimental study and discussion.....	200-220
See also Rust, orange.		relation to antecedent egg production.....	199-224
<i>Calandrinia menziesii</i> , susceptibility to curly-top.....	314	Chick-peas—	
Calcium—		heating caused by insect infestation.....	1106, 1109-1112, 1113, 1115
compounds, effect on numbers of bacteria in soil.....	1163, 1164-1165	protection by hydrocyanic-acid gas fumigation.....	649-660
effect on orange-tree growth and composition.....	278, 281, 283	See also Peas, chick.	
nitrate, effect on wheat growth.....	387-393	Chlorid, content of leaf-tissue fluids of cottons, Egyptian and Upland.....	695-704
oxid, effect on numbers of bacteria in soil.....	1163, 1164-1165	Chlorophyll deficiency in rye.....	169
California—		Chromosomes—	
beet—		literature citations.....	680-681
disease. See curly-top.		maize and maize relatives.....	673-682
leafhopper and curly-top disease.....	279-320	number in wheat, relation to morphological characters of hybrids.....	1028-1029
climate, comparison with Alabama and Japan.....	249-252	Chrysanthemum nema, <i>Apelenchus ritzeambosi</i> , description.....	1061-1062
Coachella Valley, physiography, soils, and vegetation.....	723, 725-727, 763	<i>Ciboria fruticola</i> , same as <i>Sclerotinia</i> .....	956, 957
Southern, Acala cotton, spacing.....	1081-1093	<i>Cicer arietinum</i> . See Chick-peas; Peas, chick.	
<i>Calliphora larva</i> embryology, notes.....	6, 7, 8, 10, 20	Citrus—	
Cambium curculio, <i>Conotrachelus anaglypticus</i> .....	377-386	bud variations, progeny tests and performance records.....	522-525
Campbell, F. L., and Moore, William: Studies on Nonarsenical Stomach-Poison Insecticides.....	395-402	fruits, blue mold rot prevention, results of borax treatment.....	961-968
Cane—		growth in spring, required temperatures and relation to scab.....	243-253
blight fungus of currant, comparison with blackrot of apple.....	596-597	hybrids, susceptibility to citrus canker.....	230-231, 235-239
sugar, root disease, relation to snails.....	969-970	plants, scab infection, conditions essential.....	243-253
Cane-blight fungus of currant, on currant and apple.....	584, 589-598	scab—	
Canker, citrus, susceptibility of citrus species, hybrids, and relatives.....	227-239	cause, history, and distribution.....	241-242
Canning, sweet corn, relative merits of varieties, etc.....	403-443	relation to environmental factors.....	241-254
Carbon—		yearly prevalence, conditions determining.....	249-250
disulphid, use in control of bean weevils.....	347-355	susceptibility to citrus canker.....	227-239
tetrachlorid, anthelmintic efficiency in treatment of foxes.....	331-337	wild relatives, susceptibility to citrus canker.....	227-229, 232
Carpellate hemp plant, description.....	1068	<i>Cladosporium citri</i> , cause of citrus scab, environmental factors.....	241-254
Carsner, Eubanks, and Stahl, C. F.: Studies on Curly-top Disease of the Sugar Beet.....	297-320	Clarification methods in sugar determination, comparison.....	481-483
Caryl, R. E.: Bud Selection as Related to Quality of Crop in the Washington Navel Orange.....	521-526	Clear-cut method of cutting Douglas fir, effect on reproduction.....	1233-1235, 1239-1241
<i>Casimiroa edulis</i> , susceptibility to citrus canker.....	227	Clearings, forest, effect on conditions of site.....	1149-1151
Castle, W. E., formula for estimating genetic factors.....	1098	Club-leaf, cotton growth disorder.....	810
Caterpillar, tent, insecticides testing.....	395-402	Clubroot—	
Cats, feeding with gossypol.....	191-196	effect of soil temperature and soil moisture.....	549-552, 559-561
Cats-claw, indicator of land value.....	112, 122	literature relating to.....	549-552, 559-561
Cattle—		Clustering, cotton growth disorder.....	806-807
testing for abortion infection.....	613-614, 617-620	Coachella Valley, California, cotton spacing.....	1081-1093
vaccination, control of infectious abortion.....	609-620	<i>Coix lachryma jobi</i> , pollen counts and chromosomes.....	677
<i>Ceanothus cuneatus</i> , indicator of land value.....	116, 118, 120, 123, 126	Coleogyne, indicator of land value.....	110, 118, 121, 126, 127
Cecil clay loam subsoil, colloids from, breaking strength.....	511, 512	Colloids—	
Cedar, reproduction after logging.....	1152-1154, 1157	literature cited.....	513
Cells—		soil—	
excretory, bee larva.....	1202	binding power, determination.....	506-508
sap density, relation to environmental conditions in the Wasatch Mountains, Utah.....	845-907	binding power, factors influencing.....	499-513
Cereal rusts, physiological races, investigations and discoveries.....	705	binding power of different kinds.....	511
<i>Chaetospermum glutinosum</i> , susceptibility to citrus canker.....	228	heat of wetting.....	927-935
Chamiso, indicator of land value.....	101, 113, 116, 118, 121, 122, 125, 115,	Color—	
Chaparral, indicator of land value.....	119, 120, 125, 126	picramate, dilution, effect.....	481
Chemicals, use on citrus fruits to prevent rot.....	961-963	standards for sugar determination method.....	479-480, 486
<i>Chenopodium</i> spp., susceptibility to curly-top.....	314-315	Coloration, meadow mice.....	980-982, 1014
Chickens—		Colorimeter readings, values, factors for obtaining.....	483-484
breeding, experimental study of sex ratio.....	200-220	Community plants for hot-water treatment of grain.....	79-80
flesh, vitamin B content, studies and experiments.....	463, 466-467, 468-469, 472	Composite plants, subject to nematode galls.....	474
infection with fowl typhoid, studies and post-mortem findings.....	76-77	Compounds, nitrogenous, effect on bacteria in soils.....	1159-1162
		Conifer timber, indicator of land value.....	116, 120, 123-126
		<i>Conotrachelus</i> —	
		<i>anaglypticus</i> —	
		distribution, description, life history, and control.....	377-386
		See also Curculio, Cambium.	
		spp., literature concerning.....	386
		Cook, O. F.: Acromania, or "Crazy-top," a Growth Disorder of Cotton.....	803-828
		Copper, determination in meats.....	340

	Page		Page
Corethra larvae, notes.....	7, 10, 12	Cotton—Continued.	
Corn—		yield, comparison of Pima and Upland	
best canning stage.....	434	varieties.....	948-950
canning—		yields in spacing experiments.....	1082-1093
in quality tests, method.....	431	Cottons, leaf tissue, chlorid content, compari-	
literature, review and list.....	403-406, 440-443	son of Egyptian and Upland.....	695-704
quality tests.....	431-433	Cottonseed—	
characters, correlation.....	1095-1102	feeding tests, experiments with gossypol.....	173-198
chromosome—		field germination, experiments.....	288-290
number.....	673, 678-679	germination.....	285-292
number, comparison with related plants.....	673-682	gossypol content, variation with locality.....	173
ear molds, literature citations.....	921-922	kernels, gossypol extraction and prepara-	
ears, development in different varieties.....	412-414	tion.....	173-174
field, comparison with sweet corn in compo-		laboratory germination, reports.....	290
sition and canning value.....	425,	poisoning, feeding experiments.....	173-198
427, 428, 432, 435, 438		"ensitive" samples, reasons for poor ger-	
hybrids, characters, inheritance and correla-		mination.....	286-291
tion.....	1097-1101	toxicity, relation to gossypol content.....	173-189
injury by fungus <i>Fusarium moniliforme</i> .....	909,	Couch, J. F.: Ascaris Sensitization.....	577-582
910, 918		Coville, Frederick V.: Spiny-fruited Goose-	
kernel, toughness tests.....	414-422, 432, 438	berry from Florida.....	71-74
maturity, relation to quality of canned		Cowpeas—	
product.....	409-440	heating by insects.....	1114
silking period aid in determining maturity.....	409-411	silage, composition.....	258
sugar content, relation to sweetness.....	434	weevil infestation and fumigation.....	347-356
sweet—		Cows, immunization experiments for bovine	
chemical studies, analysis methods, and		abortion, detailed records.....	633-635, 641-642
discussion of results.....	418-431	Craig, W. T., and Love, H. H.: The Genetic Relation Between <i>Triticum</i>	
varieties for canning, relative merits, etc.....	403-443	<i>dicoccum dicoccoides</i> and a Similar Morpho-	
varieties, improvement by breeding.....	435-436	logical Type Produced Synthetically.....	515-520
varieties, analyses.....	424-425	The Inheritance of Pubescent Nodes in a	
See also Maize.		Cross Between Two Varieties of Wheat.....	841-844
Cornstarch juice, sugar determination, picric		Cram, Eloise B.: A New Nematode, <i>Cylindro-</i>	
acid method.....	479-488	<i>pharynx ornata</i> , from the Zebra, with Keys	
Cotton, R. T., and Back, E. A.: Effect of Fumigation Upon Heating of		to Related Nematode Parasites of the	
Grain Caused by Insects.....	1103-1116	Equidae.....	661-672
Effective Use of Hydrocyanic-acid Gas in		<i>Craterostomum</i> spp., parasites of the Equidae.....	666, 668
the Protection of Chick-peas Warehoused		Crazy-top, cotton—	
in 240-Pound Sacks.....	649-660	diagnosis.....	812
Relative Resistance of the Rice Weevil and		disease.....	803-828
the Granary Weevil to High and Low		irregular occurrence and different condi-	
Temperatures.....	1043-1044	tions of soil.....	819-821
Cotton, R. T., and Brooks, Fred E.: The		symptoms and effects, comparison with	
Cambium Curculio <i>Conotrachelus anaglyp-</i>		other disorders.....	813-819
<i>ticus</i> .....	377-386	Creatinin, determination method.....	340
Cotton—		Creech, G. T., and Buck, J. M.: Studies Re-	
abnormal branching, effects of crazy-top		lating to the Immunology of Bovine Infec-	
and other diseases.....	813-814	tious Abortion.....	607-642
Acala, spacing experiments in Southern		Creosote bush, association on Southwestern	
California.....	1081-1093	desert soils.....	733-745, 795-799
acromania or "crazy-top".....	803-828	Creosotebush, indicator of land value.....	111, 112,
bolls—		118, 120-127	
injury by cambium curculio.....	379, 385	<i>Crepis</i> spp., seed infestation with nematodes.....	474, 475
shedding and development, comparison.....	943-954	Cross-breeding, wheat, for velvet nodes.....	841-844
breeding and growth disorders.....	824	Crucifers, clubroot disease, effect of soil tem-	
Egyptian, chlorid content of leaf, compari-		perature and soil moisture.....	549-562
son with Upland cotton.....	695-704	<i>Cryptolestes pusillus</i> heating of grain.....	1105, 1115
fiber, salt content.....	293-295	Culpepper, C. W., and Magoon, C. A.: Stud-	
flowers and bolls, comparison of Pima with		ies Upon the Relative Merits of Sweet Corn	
Upland.....	941-952	Varieties for Canning, etc.....	403-443
growing on saline soils, relation to chlorid		Culture media for <i>Bacillus larvae</i> , and use.....	135-141
content.....	696, 701	Cultures, <i>Bacterium abortus</i> , use as vaccine to	
growth, irregularities caused by crazy-top		immunize cattle.....	609-620
and other diseases.....	813-819	Curculio—	
heredity, relation to growth disorders.....	822-823	cambium.....	377-386
irrigated, spacing experiments in southern		cambium, enemies and control methods.....	385, 386
California, 1922 and 1923.....	1081-1093	Curly-leaf, beet. See Curly-top.	
lint and seed characters, comparisons.....	950-952	Curly-top—	
overwintering, study for disease spread.....	825	beet—	
Pima—		distribution and the beet leafhopper.....	299-300
comparison with Upland varieties in		economic importance.....	300-302
Arizona.....	938-954	causative organism.....	312
terminal bud abortion, noninheritance.....	1041-	disease—	
1042		incubation period in insects and in plants.....	306-
plants, dwarfing, effects of crazy-top and		307, 312	
other diseases.....	813	sugar beets, studies.....	297-320
Upland—		effect on growth and weight of beets.....	303-304
chlorid content of leaf, comparison with		virus—	
Egyptian cotton.....	695-704	overwintering.....	315
varieties, comparison with Pima cotton		transmission methods and factors affect-	
in Arizona.....	938-954	ing.....	306-316
varieties—		Currant—	
susceptibility to crazy-top and other		cane blight fungus, on currant and apple....	584,
growth disorders.....	821-822	589-598	
variation of gossypol content in seed.....	177-178,	<i>Physalospora malorum</i> occurrence.....	583-588
187		Currants, black, spread of blister-rust in-	
		fection.....	1253, 1255, 1257

	Page		Page
Cyanamid—		Dunegan, John C., and Roberts, John W.: The Fungus Causing the Common Brown Rot of Fruits in America.....	955-960
and dicyanodiamid nitrogen, determination method.....	42-43	Durango cotton, growing and characters, comparisons.....	938-952, 954
bibliography.....	68-69	Dusts, insoluble aromatic compounds, in- secticidal value.....	401
effect on microorganisms in soil.....	1159-1166	Dycyanodiamide, fertilizer tests.....	973-975
fertilizer tests.....	973-975	Ear—	
nitrification rates, comparisons.....	43-54	corn, development in different varieties..	412-414
transformation products, changes in soil.....	54-66	length, meadow mouse.....	988
Cyanids, insecticidal value.....	396-399	Earths, alkaline determination in meats.....	341
<i>Cylicobrachytus</i> spp., parasites of the Equidae.....	669	<i>Eberthella sanguinaria</i> , same as <i>Bacterium</i> <i>sanguinarium</i> .....	75, 77
<i>Cylicodontophorus</i> spp., parasites of the Equidae.....	670	Edema, caused by gossypol poisoning.....	195
<i>Cylicostephanus</i> spp., parasites of the Equidae.....	671	Eelworms. See Nematodes.	
<i>Cylicostomum</i> spp., parasites of the Equidae.....	666, 668, 670, 672	Egg production—	
<i>Cylicotoichus</i> sp., parasite of the Equidae.....	669	relation to the sex ratio of—	
<i>Cylindropharynx</i> spp., parasites of the Equidae.....	666, 668, 672	chickens.....	207-209
<i>Cylicocercus</i> spp., parasites of the Equidae.....	669	the domestic fowl.....	199-224
<i>Cyclorhapha</i> , larvae.....	12, 14	Eggs—	
Cyrtosis, cotton growth disorder.....	810	consumption, per capita.....	461
Dairy wastes, aromatic bacillus.....	275-276	Vitamin content.....	461-472
Dandelion—		weight, relation to sex ratio of chickens..	209-211
false, infestation with nematode of <i>Tylenchus</i> <i>dipsaci</i> .....	473-477	Egyptian cotton, chlorid content of leaf tissue, comparison with Upland.....	695-704
infestation with nematode, <i>Tylenchus</i> <i>dipsaci</i> .....	473, 475-477	Embryos, meadow mouse, number and weight.....	989
Davison, F. R. and—		Emmer, wheat group, characters.....	1017
Brewbaker, H. E., and Thompson, N. A.: Brittle Straw and Other Abnormalities in Rye.....	169-172	Emmers—	
Willaman, J. J.: Some Modifications of the Picric Acid Method for Sugars.....	479-488	comparison of synthetic form with wild form.....	515-520
Day length—		wild, study and comparisons.....	515-520
effect on internodal length and leaf dimen- sions.....	453-460	<i>Encelia farinosa</i> , indicator of land value.....	112, 122
relation to growth and sexual development of hemp.....	1069-1076	<i>Endophyllum</i> spp., characteristics.....	1050, 1052, 1053
Daylight length, artificial control, effect on blossoming and growth.....	447-460	Ensiling Sudan grass with a legume.....	255-259
Dearstyne, R. S., and Kaupp, B. F.: Fowl Typhoid, Its Dissemination and Control.....	75-78	Epiornithological studies of fowl typhoid.....	78
Delmas, pecan susceptibility to scab.....	322, 328	Equidae, nematode parasites.....	666-672
Delphinium, bacterial leafspot.....	261-270	<i>Eremocitrus glauca</i> , susceptibility to citrus canker.....	228
Demaree, J. B.: Pecan Scab, with Special Reference to Sources of the Early Spring Infections.....	321-329	<i>Eristalis</i> sp., larvae.....	8, 10, 13, 20
Denmark, clubroot studies in.....	550-552	<i>Erodium cicutarium</i> . See Alfalfa.	
Desert region, southwestern, natural vegeta- tion, indicator significance.....	721-802	Ether extract, determination in meats.....	340
Desert-sage, association on Southwestern desert soils.....	745-757, 796-799	<i>Euchlaena</i> spp., chromosome number, pollen counts, etc.....	673-676
Dessication, effect on virus of curly-top.....	311	<i>Eutettix tenella</i> . See Leafhopper, beet.	
Devarda alloy, use in nitrate determina- tion.....	527, 528-538	Fasciation, cotton, effects of crazy-top and other diseases.....	814
Diarrhea, caused by gossypol.....	192	Fat—	
Dicyanodiamid, nitrification in soils and effects on fertilizers.....	39-40, 54-61	cells, bee larva, description.....	1200-1201
Dimorphism, sexual, of hemp, factors affect- ing.....	1067-1078	tissue of apple maggot and other larvae....	16-19
<i>Diplodia natalensis</i> , growing on apple and currant twigs.....	587	Feeding—	
<i>Dipsacus fullonum</i> , seed infestation with nematodes.....	474, 477	cottonseed, experiments with gossypol....	173-198
Diptera, anatomy, embryology, and meta- morphosis.....	6, 7, 8, 12, 20, 28, 30, 31	pigeons, with poultry flesh and eggs.....	462-471
Dodge, B. O.—		<i>Feronia</i> spp., susceptibility to citrus canker.....	228
and Stevens, Neil E.: The Rhizoctonia Brown Rot and Other Fruit Rots of Strawberries.....	643-648	Fertilizers—	
Expulsion of Aecidiospores by the May- apple Rust, <i>Puccinia podophylli</i> .....	923-926	nitrate determination methods.....	533-538
Uninucleated Aecidiospores in the <i>Caeoma</i> - <i>nitens</i> and Associated Phenomena.....	1045-1058	value of phosphorus nitride and ammonium sulphate, comparison.....	1118
Donkey, parasites.....	666-672	Fescue, indicator of land value.....	107, 117, 122
Dormant spray for pecan scab.....	327	Fiber, cotton, salt content.....	293-295
<i>Dorylaimus regius</i> , description, habits, and food.....	1062-1064	Fir, Douglas—	
<i>Dothiorella pycnidia</i> , from currant cane blight fungus, etc.....	590, 591	cutting methods, effect on reproduction	1233-1242
Drummond, Pearl L., and Toole, Eben S.: The Germination of Cottonseed.....	285-292	reproduction—	
Dry farming land, indications of natural vegetation.....	117	after logging.....	1152-1154, 1157
Ducks, flesh, vitamin B content.....	463, 468, 472	on pine lands of central Idaho.....	1140-1147
Duff, moisture and temperature fluctuations, comparison with bare soil.....	1154-1156	under various cutting methods.....	1233-1242
		Fixed Nitrogen Research Laboratory, fertil- izer experiments.....	971-976
		Flats, bare, southwestern desert soils.....	779
		<i>Flavobacterium suaveolens</i> , a new species of aromatic bacillus from dairy wastes.....	275-276
		Florida, gooseberry, spiny-fruited, native species.....	71-74
		Flowers, cotton, abnormalities, effects of crazy-top and other diseases.....	818
		Fly, apple maggot—	
		anatomy and metamorphosis, notes, and bibliography.....	1-36
		technical description.....	21-30, 32
		Foot—	
		meadow mouse, hind, measurement.....	987
		meadow mouse, plantar tubercles vari- ation.....	983, 1014
		Ford, E. R., and Posey, G. B., Survey of Blister Rust Infection on Pines at Kittery Point, Me., etc.....	1253-1258



	Page		Page
Forest—		Godfrey, G. H.: Dissemination of the Stern and Bulb Infesting Nematode <i>Tylenchus dipsaci</i> , in the Seeds of Certain Composites.....	473-478
distribution, relation to sap density.....	892-895	Godkin, James, Johnson, A. G., and Reddy, C. S.: Bacterial Blight of Rye.....	1039-1040
reproduction—		Gonads, rudiments in bee larva.....	1202-1203
after logging in northern Idaho.....	1149-1157	Gooseberries, eradication for blister rust unnecessary in Southeast.....	74
injury by grazing and other causes.....	1145-1146	Gooseberry, spiny-fruited, from Florida.....	71-74
<i>Fortunella</i> —		Gossypol—	
<i>hindii</i> , susceptibility to citrus canker.....	228	content of cottonseed, relation to toxicity.....	173-189
spp., susceptibility to citrus canker.....	228	extraction from cottonseed and preparation.....	173-174
Foulbrood—		intrapertitoneal injection into animals.....	174-178
causes and development.....	130-133	literature concerning.....	188-189, 197
European, organisms associated with.....	129,	pharmacology.....	191-198
130, 132, 135		Gradus peas, rogue type, crossing and backcrossing.....	1247-1252
relation to the metabolism of its causative organism.....	129-168	Grain—	
Fowl—		fumigation, effect on heating caused by insect infestation.....	1103-1116
domestic. See Chicken.		heating in storage, causes.....	1108-1114
typhoid, dissemination and control.....	75-78	insects, control in warehouses, experiments with chick-peas.....	649-660
Fowls—		land, indications of natural vegetation.....	117
infected with typhoid, description, symptoms, and results.....	76, 77	pests, literature citations.....	1116
vaccination for control of typhoid.....	78	treatment with hot water, community plants.....	79-80
Foxes, treatment with carbon tetrachlorid.....	331-337	Gramma grass, indicator of land value.....	100,
Frederich, William J., and Peltier, George L.: Further Studies on the Relative Susceptibility to Citrus Canker of Different Species and Hybrid of the Genus Citrus, etc.....	227-239	103, 104-105, 106, 117-122	
Relation of Environmental Factors to Citrus Scab Caused by <i>Cladosporium citri</i> Massee.....	241-254	Granary weevil, resistance to high and low temperatures.....	1043-1044
Freezing, soil, limiting factors in sap density of plants.....	894	Grapefruit—	
Fruit, sound, immunity to cambium curculio.....	378, 379	scab infection, temperature limits.....	243
Fruits, brown rot, fungus causing.....	955-960	treatment with borax to prevent rot.....	965, 968
Fulton, Harry R., and Bowman, John J.: Preliminary Results with the Borax Treatment of Citrus Fruits for the Prevention of Blue Mold Rot.....	961-968	Grasses—	
Fumigation—		indicators of land value.....	101-107, 112-114, 117-127
effect upon heating of grain caused by insects.....	1103-1116	inoculation with rust, <i>Puccinia graminis poae</i> .....	541-543
effects on bean weevils, adults, eggs, and forms within seeds.....	348-355	Grazing—	
grain warehouses, experiments with chick peas.....	650-659	injury to conifer reproduction.....	1145, 1146
Fungi, sporocarps, relation of size and structure to host.....	586-587	land, indications of natural vegetation.....	118
Fungus—		lands, carrying capacity of vegetation.....	119-121
brown rot, literature.....	959-960	Greasewood, indicator of land value.....	110,
causing brown rot of fruits in America.....	955-960	118, 120, 125, 126	
Fusarium—		Greenhouse—	
bulb rot of onion, and relation of environment to development.....	683-694	experiments with atmospheric nitrogen fertilizers and related compounds.....	971-976
<i>cepae</i> —		onion rot, studies and experiments.....	687-688
cause of onion bulb rot, description, etc.....	684-688	Grossularia—	
reaction to temperature, acidity, and alkalinity.....	685-686	<i>curvata</i> , description, comparison with <i>G. echinella</i> .....	71-73
<i>moniliforme</i> —		<i>echinella</i> , spiny-fruited gooseberry from Florida, description, etc.....	71-74
an ascigerous stage and synonymy.....	909-922	spp., American, comparison with <i>echinella</i> .....	72-74
culture studies on conidia.....	910, 913-916	Guanidin—	
<i>Fusicladium effusum</i> , cause of pecan scab, nature and control.....	324-328	nitrate, effect on numbers of bacteria in soil.....	1165, 1166
Gaines, E. F., and Sax, Karl: A Genetic and Cytological Study of Certain Hybrids of Wheat Species.....	1017-1032	nitrification in soils.....	40, 63, 65-66
Galleta, indicator of land value.....	106, 118, 119, 125	Guanylurea—	
Galls, nematode, occurrence on composite plants.....	473-477	effect on numbers of bacteria in soil.....	1161,
Gemete production in certain crosses with "rogues" in peas.....	1247-1252	1165, 1166	
Genetics, wheat, literature.....	1031-1032	fertilizer tests.....	973-975
Germination—		nitrification in soils and rate of decomposition.....	40, 56-58, 62, 64
cottonseed.....	285-292	Guinea—	
seed, western white pine, by seasons.....	1127-1 28	fowl, flesh, vitamin B content.....	463, 468, 472
western white pine, relation to surfaces.....	1151-1154	pigs, inoculation with milk from diseased cows.....	622-642
wheat, effect of modified hot-water treatment.....	80-89	<i>Gyalocephalus</i> spp., parasites of Equidae.....	666, 667
Gestation, meadow mouse, period and number of young.....	989	<i>Gymnoconia interstitialis</i> , spore discharge from acidia.....	925
Gibberella—		Haas, A. R. C., and Reed, H. S.: Some Relations Between the Growth and Composition of Young Trees and the Concentration of the Nutrient Solution.....	277-284
<i>acervalis</i> , similarity to <i>Fusarium moniliforme</i> .....	917	"Hairy neck"—	
<i>moniliformis</i> , technical description.....	920	character of rye, investigations.....	568-571
<i>sabuinetti</i> , similarity to <i>Fusarium moniliforme</i> .....	914	wheat segregates from wheat-rye hybrids.....	567-576
Glands, silk, bee larva.....	1189-1190, 1207	Hanson, Karl B., and Van Volkenberg, H. L.: Anthelmintic Efficiency of Carbon Tetrachlorid in the Treatment of Foxes.....	331-337
Glycogen, utilization in metabolism of <i>Bacillus</i> larvae.....	151-153	Hardiness, plant, relation to sap density.....	898-899
		Hare, European—	
		habits, control, and value for food and sport.....	1133-1137
		in North America.....	1133-1137

	Page		Page
Hares, European, introduction, distribution, and abundance.....	1134-1136	Hydrocyanic-acid gas—Continued.	
Harris, J. Arthur: The Chlorid Content of the Leaf-Tissue Fluids of Egyptian and Upland Cotton.....	695-704	use in—	
Harrison, W. T.: Ascaris Sensitization.....	577-582	control of bean weevils.....	347-355
Hartsville cotton, growing and characters, comparisons.....	938-952, 954	protection of chick-peas in 240-pound sacks.....	649-660
Head, meadow mouse—		<i>Hypochaeris radicata</i> . See Dandelion, false.	
and development of skull.....	989-992	<i>Hypodermis</i> —	
measurement and body.....	985-987	<i>bovis</i> , second-stage larva, comparison with <i>H. lineatum</i> .....	274
Heart—		<i>lineatum</i> , larval stages, comparison of characters.....	271-274
bee larva, and blood cells.....	1195-1200, 1207	Idaho—	
enlargement, caused by gossypol.....	196	central, growth on cut-over and virgin western yellow pine lands.....	1139-1148
Hearts, composition.....	344	northern, reproduction after logging, some factors affecting.....	1149-1157
Heat—		Priest River Forest Experiment Station, pine-seed sowing.....	1127-1131
effect on virus of curly-top.....	311	Imaginal buds, apple-maggot larvae.....	7-8
in stored grain, caused by insect infestation.....	1103-1114	Indiana, wheat treating in community plants.....	79-80
wetting, of soil colloids.....	927-935	Inoculation—	
Heifers, immunization experiments for bovine abortion.....	621-633, 636-641	oats with stem rust to test varietal resistance, results.....	710-718
Hemlock, reproduction after logging.....	1152-1154, 1157	onion with <i>Fusarium</i> bulb rot, greenhouse experiments.....	687-688
Hemp—		Insect balls in grain, notes.....	1109
flower mutilation, effect on sex development.....	1077-1078	Insecticides—	
growth, relation to length of daylight.....	1070-1074	nonarsenical stomach-poison, studies.....	395-402
sex, influence of environment.....	1067-1080	testing—	
Hens, flesh, vitamin B content.....	463, 466-467, 468-469, 472	method.....	395
Herbs, Waastch Mountains, freezing point, depressions, and osmotic pressures.....	871-875, 880-884	on insects and on bean foliage.....	395-402
Herd, bovine abortion, vaccine treatment.....	617-620	Insects—	
<i>Hesperethusa crenulata</i> , susceptibility to citrus canker.....	228	carriers of crazy-top disease, study.....	825
Hessian fly—		cause of heating in grain.....	1103-1114
parasite <i>Platygaster vernalis</i> —		forest, losses caused to pine and Douglas fir.....	1144
development and feeding.....	833-837	number, relation to curly-top and variation of individuals.....	307-310
polyembryonic development.....	829-840	Internodal length, effect of varying day length.....	453-460
<i>Hilaria rigida</i> , indicator of land value.....	106, 118, 121	Internodes, cotton, irregularity, effects of crazy-top and other diseases.....	815
Hill, C. C., and Leiby, R. W.: The Polyembryonic Development of <i>Platygaster vernalis</i> .....	829-840	Involucres, abnormal, of cotton, effects of crazy-top and other diseases.....	817
Hip glands, meadow mouse, variations.....	984-985, 1014	Iodin, permeability gradient in wheat seed coats.....	225-226
Hoagland, Ralph, and—		Iron determination in meats.....	341
Lee, Alfred R.: Antineuritic Vitamin in Poultry Flesh and Eggs.....	461-472	Ironwood, indicator of land value and possibilities.....	112, 122
Powick, Wilmer C.: The Chemical Composition of Edible Viscera From Meat-producing Animals.....	339-346	Jackson, H. S., and Mains, E. B.: Aerial Stages of the Leaf Rusts of Rye and of Barley in the United States.....	1119-1126
Holding tests, citrus fruits treated with chemicals for prevention of rot.....	961-963	Jacob, K. D.: Chemical and Biological Studies with Cyanamid and Some of its Transformation Products.....	37-69
Honeybee—		Jala maize, crossing with Tom Thumb maize, results.....	1095-1102
cluster, heat production.....	1109	Japan, climate, comparison with Alabama and California.....	249-252
fat tissue in larvae and in mature insects.....	16, 17, 18, 19	Job's-tears, pollen counts and chromosome number.....	677
larva, morphology.....	1167-1214	Johnson, A. G., Reddy, C. S., and Godkin, James: Bacterial Blight of Rye.....	1039-1040
Hookworm, fox, treatment with carbon tetrachloride.....	331-337	Jull, M. A.: The Relation of Antecedent Egg Production to the Sex Ratio of the Domestic Fowl.....	199-224
Hopperdozer, use in control of leafhopper.....	316	Kaupp, B. F., and Dearstyne, R. S.: Fowl Typhoid, its Dissemination and Control.....	75-78
Horse—		Kearney, Thomas H.—	
Ascaris, fluid extract eye-test experiments on horses.....	577, 580, 581, 582	Non-inheritance of Terminal Bud Abortion in Pima Cotton.....	1041-1042
parasites.....	666-672	The Salt Content of Cotton Fiber.....	293-295
Hot-water treatment, injury to wheat, relation of seedcoat protection.....	86-89	Kempton, J. H.: Correlation Among Quantitative Characters in Maize.....	1095-1102
Howell, A. Brazier: Individual and Age Variation in <i>Microtus montanus yosemitae</i> .....	977-1016	Kernel, corn, toughness tests.....	414-422, 432, 438
Humus depth, relation to root length of forest seedlings.....	1238-1239	Kidneys, composition.....	344
Hybosis, cotton growth disorder.....	809-810	<i>Kigelia pinnata</i> , host plant of <i>Heterodera radiculicola</i> .....	1061
Hybrid—		King, C. J.: Comparison of Pima Cotton with Upland Varieties in Arizona.....	937-954
maize, Jala and Tom Thumb, character inheritance, etc.....	1097-1101	Korstain, C. F.—	
teosinte-and-corn, pollen counts and chromosomes.....	674-676	Density of Cell Sap in Relation to Environmental Conditions in the Wasatch Mountains of Utah.....	845-907
Hybrids—		Growth on Cut-over and Virgin Western Yellow Pine Lands in Central Idaho.....	1139-1147
citrus, susceptibility to citrus canker.....	230-231, 235-239		
wheat species, study, genetic and cytological.....	1017-1032		
wheat-rye, "hairy neck" segregates.....	567-576		
Hydrocyanic-acid gas—			
fumigation of grain for insect pests.....	1106, 1113, 1114, 1115		

	Page		Page
Kubanka—		Livers—	
durum wheat, crosses with black winter		antineuritic value.....	469, 472
emmer.....	516-519	composition, table.....	342-343
wheat, crosses with <i>vulgare</i> species.....	1019-1031	Logging, effect on forest cover and reproduc-	
Kumquats, resistance to citrus canker.....	228	tion.....	1149-1157
Laake, E. W.: Further Observations on the		Lone Star cotton, growing and characters,	
Molts of the Ox Bots, <i>Hypoderma bovis</i> De		comparisons.....	938-952
Geer and H. <i>Lineatum</i> Villers.....	271-274	Longley, Albert E.: Chromosomes in Maize	
Land, agricultural potentiality characterized		and Maize Relatives.....	673-682
by plant associations.....	800-801	Loomis, H. F.: Comparison of Pima Cotton	
Larch, reproduction after logging....	1152-1154, 1157	with Upland Varieties in Arizona.....	937-954
Larkspur—		Louisiana, sugar-cane root disease, relation	
disease, bacterial leaf spot, cause and con-		to snails.....	969-970
trol.....	261-270	Love, H. H., and Craig, W. T.: The Genetic Relation Between <i>Triticum</i>	
inoculation with leafspot bacteria.....	263-264	<i>dicoccum dicoccoides</i> and a Similar Morph-	
Larsen, J. A.: Some Factors Affecting Repro-		ological Type Produced Synthetically... 515, 520	
duction After Logging in Northern		The Inheritance of Pubescent Nodes in a	
Idaho.....	1149-1157	Cross Between Two Varieties of Wheat. 841-844	
Larson, A. O.: Fumigation of Bean Weevils		<i>Lyctus planicollis</i> . See Beetle, powder-post;	
<i>Bruchus obtectus</i> and <i>B. quadrimaculatus</i> .....	347-356	Powder-post beetle.	
Larva, bee, description, anatomy, and his-		Mackie, William W., and Allen, Ruth F.: The Resistance of Oat Varieties to Stem	
tology of various parts.....	1167-1208	Rust.....	705-720
Larvae—		Maggot, apple anatomy and metamorphosis... 1-36	
apple maggot, development.....	1-21	Magnesium sulphate, effect on wheat growth.....	387-393
bee, composition at different ages.....	142-150	Magnesium-oxid method of nitrate deter-	
<i>Lasioderma serricorne</i> , control in chick-peas. 649, 658		mination, comparisons.....	536-538
Lawrence—		Magoon, C. A., and Culpepper, C. W.: Stud-	
John V.: The Chlorid Content of the Leaf		ies Upon the Relative Merits of Sweet Corn	
Tissue Fluids of Egyptian and Upland		Varieties for Canning, etc.....	403-443
Cotton.....	695-704	Mahogany, mountain, indicator of land value	
Zonia Wallen: The Chlorid Content of the		and possibilities.....	115, 118, 120, 123, 124, 125
Leaf Tissue Fluids of Egyptian and Up-		Maine—	
land Cotton.....	695-704	Experiment Station, wheat hybrids, grow-	
Lead arsenate, insecticidal value, comparison		ing experiments.....	1019
with cuprous cyanid.....	399	Kittery Point, pines' infection with blister	
Leaf—		rust, etc., survey.....	1253-1258
dimensions, relation to varying day		Mains, E. B., and Jackson, H. S.: Aerial	
length.....	453-460	Stages of the Leaf Rust of Rye and of Bar-	
forms, cotton, abnormal, effects of crazy-top		ley in the United States.....	1119-1126
and other diseases.....	815-816	Maize—	
orange, sap concentration and reaction,		characters, correlation.....	1095-1102
relative constancy.....	277	See also Corn.	
Leaf-curl, cotton, growth disorder.....	809-810	Malpighian tubules, bee larva.....	1188-1179, 1207
Leaf-cut, cotton growth disorder.....	807-809	<i>Maka parviflora</i> , susceptibility to curly-top... 315	
Leafhopper—		Mammals, small, preparation of skulls for	
beet—		collection.....	978-979
control method.....	316-317	Mammæ, meadow mice.....	983, 1014
distribution.....	299-300	Mandible, meadow mouse, description and	
transmission of curly-top disease.....	306-310	variation.....	1009, 1015
curly-top virus incubation period.....	306-308	Map, western United States, key to vegeta-	
Leafspot, bacterial, of Delphinium.....	261-270	tion regions.....	100, 123-127
Leather rot—		Marquis wheat crosses with emmer spe-	
of strawberries, cause and description.. 646-648		cies.....	1019-1031
strawberry.....	357-376	Matchweed, indicator of land value and pos-	
Leaves—		sibilities.....	101, 103, 104, 119-125
conifer, sap density seasonal variations.. 889-892		Maturity, corn, tests for canning stage.....	434
cotton, discoloration, effect of mosaic dis-		Mayapple rust, expulsion of aecidiospores. 923-926	
eases.....	816-817	McClelland, T. B.: The photoperiodism of	
pecan, scab description and source of in-		<i>Tephrosia candida</i> .....	445-460
fection.....	322-323, 325	McKeever, H. G.: Spacing Experiments with	
young orange trees, composition.....	282, 283	Acala Cotton in Southern California... 1081-1093	
Lee, Alfred R., and Hoagland, Ralph: Anti-		McPhee, Hugh C.: The Influence of Environ-	
neuritic Vitamin in Poultry Flesh and		ment on Sex in Hemp, <i>Cannabis sativa</i> ... 1067-1080	
Eggs.....	461-472	Meadows, vegetation type, indicator of land	
Legumes, sowing with Sudan grass for silage		value.....	108
mixture.....	256-257	Meat, hare, food value and quantity, esti-	
Leiby, R. W., and Hill, C. C.: The Poly-		mate.....	1137
embryonic Development of <i>Platygaster</i>		Mebane cotton, growing and characters, com-	
<i>ternalis</i> .....	829-840	parison.....	938-952
Leighty, Clyde E., and Taylor, J. W.: Hairy		<i>Melophagus ovinus</i> , embryology studies. 6, 10-11, 13	
Neck Wheat Segregates from Wheat-Rye		Mesquite—	
Hybrids.....	567-576	association with chamiso on southwestern	
Lemons, treatment with borax to prevent rot,		desert soils.....	766-769, 796-799, 800
experiments.....	962-968	indicator of land value and possibilities... 103,	
Leonard, Lewis T.: Effect of Mositure on		thicket on southwestern desert soils.....	757-759
Seed-borne Bean Disease.....	489-497	Metals, alkali, determination in meats.....	341
<i>Lepus europæus</i> , in North America.....	1133-1137	Mice, meadow—	
Levine, M. N., and Stakman, E. C.: <i>Puccinia</i>		characters, variations with sex and age... 977-1016	
<i>graminis poae</i> Erikss. and Henn. in the		male and female, measurement..... 981-982, 1014	
United States.....	541-548	measurement, methods employed and	
Light, relation to curly-top infection.....	310	results.....	979, 985-988, 1014
Limbs, variations, progeny tests.....	522-523	Microcitrus, susceptibility to citrus canker. 228, 230	
Lime content of cyanamid, relation to increase		Microorganisms in soil, effect of cyanamid	
of soil bacterial numbers.....	1163-1164	and related compounds.....	1159-1166
Lime-sulphur wash, as repellant on orchard			
trees.....	1137		
Liming soil, effect on clubroot.....	551, 558		
Lint cotton, comparisons.....	950-952		

	Page		Page
<i>Microtus montanus yosemitae</i> —		Nitrogenous—	
variation, individual and age.....	977-1016	compounds, analysis, methods.....	41-43
See also Mouse, meadow; Mice, meadow.		materials, nitrate and ammonia determina- tion.....	527-539
Middleton, Howard E.: Factors Influencing the Binding Power of Soil Colloids.....	499-513	Nodes, pubescent, inheritance in a cross be- tween two varieties of wheat.....	841-844
Milk, infection with abortion bacteri.....	614, 620	Nolina, indicator of land value and possi- bilities.....	114, 118, 120, 123
Minnesota rye, abnormalities occurrence.....	169	Nonarsenical stomach-poison insecticides.....	395-402
Mistletoe, losses caused to Douglas fir in Idaho forests.....	1144-1145	<i>Northoscordium bivalve</i> , host of <i>Uromyces</i> <i>hordci</i> .....	1124
Mixtures, silage crops, typical combinations..	255	Nut, pecan, scab, description and source of infection.....	323, 325
Moisture—		Nutrient solution—	
content, sweet corn.....	426	concentration, relation to orange trees, growth and composition.....	277-284
cottonseed, relation to germination.....	290-291	molecular proportions, relation to wheat growth.....	387-393
determination in meats, method.....	339	Shive's formula.....	388
effect on—		Oak, lumber, steaming for control of powder- post beetle.....	1033-1038
forest reproduction.....	1235-1236	Oats—	
seed-borne bean disease.....	489-497	breeding, for resistance to stem rust, and results.....	706
relation to citrus scab infection and devel- opment.....	243-253	inoculation with stem rust, tests of resist- ance in varieties.....	706-709
Molds, corn ear, studies.....	909-922	resistant to stem rust, list of varieties.....	706
Molting, meadow mice.....	983, 1014	stem rust, resistance by oat varieties.....	705-720
Molts, ox bots, observations.....	271-274	varieties—	
Montana, Lola National Forest, Savanac nursery pine-seed sowing.....	1127-1131	inoculation with stem rust to test resist- ance, results.....	710-718
Monteith, John, Jr., Relation of Soil Tem- perature and Soil Moisture to Infection by <i>Plasmodiophora brassicae</i> .....	549-562	resistance to stem rust.....	705-720
Moore, William, and Campbell, F. L.: Studies on Nonarsenical Stomach-poison Insecticides.....	395-402	Ocotillo, indicator of land value and possi- bilities.....	112, 118, 122, 127
Morphology, honeybee larva.....	1167-1214	Oenocytes, bee larva, description.....	1201-1202
Mortality, prenatal, in chickens, relation to sex ratio.....	202-207	<i>Oesophagodontus</i> spp., parasites of Equidae, key.....	666, 667
Mountain weed, indicator of land value and possibilities.....	108, 118, 122-126	Onion—	
Mouse, meadow—		diseases, literature citations.....	693-694
coloration and other characters, variations..	980- 1014	Fusarium bulb rot, and relation of envi- ronment to development.....	683-694
skull, description, and measurement of bones and teeth.....	993-1014	seedlings, infestation with nematodes.....	477
variation, individual and age.....	977-1016	storage, relation of temperature to rot development.....	691-692, 693
Mulching strawberries for prevention of rot..	374	Onions, inoculation with Fusarium bulb rot, greenhouse experiments.....	687-688
Mules, parasites, key.....	666-672	<i>Oospora verticilloides</i> , similarity to <i>Fusarium</i> <i>moniliforme</i> .....	918-920
Mummy peas, crossing with Gradus rogue and Rice's 330.....	1247-1248	Orange—	
Muscles, bee larva, head and trunk, descrip- tion.....	1190-1195, 1207	Thompson strain, progeny propagation of limb variations, results.....	522-525
Muscolidae, anatomy, embryology, and meta- morphosis.....	8, 10, 14-15, 29, 30	trees, growth and composition, relation to nutrient solution.....	277-284
<i>Myiophasia globosa</i> , enemy of cambium curculio.....	385, 386	Valencia, effect of concentration of solu- tion on young trees.....	280-281
Needlegrass, indicator of land value and pos- sibilities.....	102-105, 117, 120, 121, 1214	Washington navel, quality of crop as re- lated to bud selection.....	521-526
Nelson, James A.: Morphology of the Honey- bee Larva.....	1167-1214	Oranges—	
Nemas, parasitic, on plants and related forms.....	1059-1066	brown-spotted, from limb variations.....	522
Nematode—		factors determining quality of crop.....	521
<i>Cylindropharynx ornata</i> , from the zebra, with keys to other nematode parasites.....	661-672	leaf sap, concentration and reaction, rela- tive constancy.....	277
new, <i>Cylindropharynx ornata</i> , of zebra.....	661-672	rind, juice, color, size, and shape, relation to quality.....	521
<i>Tylenchus dipsaci</i> , dissemination in seeds of certain composites.....	473-478	scab infection, temperature limits.....	243
Nematodes—		treatment with borax to prevent rot, ex- periments.....	961-968
literature relating to.....	478	<i>Ornithogalum</i> spp., host of <i>Puccinia anomala</i> , aecial stage.....	1124-1125
plant, literature citations.....	1065-1066	<i>Orthorrhapha</i> larvae, notes.....	12, 14
Nervous systems, bee larva, brain, ventral nerve cord, etc.....	1172-1179, 1205	Osmotic phenomena, relation to cell sap con- centration.....	846-847
Netting, poultry, use in protecting trees against hares.....	1137	Ovaries, honeybee queen larva.....	1203
Niggerwool, indicator of land value and pos- sibilities.....	100, 101, 102, 104, 118, 120-125	Ox bots, molts, observations.....	271-274
Nitrate—		Palms, growing in desert soils, Coachella Valley.....	763-764
determination in—		Paloverde, indicator of land value and possi- bilities.....	112, 122
fertilizers, comparison of methods.....	533-536	Paralysis, caused by gossypol poisoning.....	194
nitrogenous materials.....	527-539	<i>Paramignya monophylla</i> , susceptibility to citrus canker.....	228
formation from cyanamid and urea in soils.....	49-53	Parasite, Hessian fly, <i>Platygaster vernalis</i> , polyembryonic development.....	829-840
nitrogen determination method.....	42-43	Parasites—	
Nitrification of phosphorus nitride.....	1117-1118	nematode—	
Nitrogen—		literature citations.....	672
atmospheric, fertilizers, greenhouse experi- ments.....	971-976	of the Equidae, horse, donkey, mule, and zebra, key.....	666-672
determination in meats.....	340		
importance in wheat growth and develop- ment.....	393		

	Page		Page
<i>Paratylenchus nanus</i> infestation of zinnia roots.....	1064-1065	Plant—	
<i>Parosela arborescens</i> , indicator of land value and possibilities.....	113, 122	communities on desert soils, detailed description.....	730-779
Pasture, gains of steers, effects of winter rations.....	1215-1232	growth, effect of varying the day length.....	451-453
<i>Patellina fragaria</i> , cause of tan rot of strawberries.....	645	succession, relation to sap density.....	892-895
Peas—		Plants—	
crossing, gamete production.....	1247-1252	desert—	
mutations in cross breeding.....	1247-1252	associated on southwestern desert region, description.....	730-779
Pecan—		list, with botanical names.....	781
scab, and sources of early spring infections.....	321-330	dioecious, sex reversal, discussion.....	1079
trees, spraying, difficulties.....	328	food supply, seasonal variations.....	891-892
Pecans—		growth and reproduction, relation to sap density.....	895-896
growing in Southeast, and distribution of scab.....	321	semiarid, indicators of land values.....	100-127
spraying for scab-control experiments.....	326-327	structure and physiology, variation with environment.....	896-898
varietal susceptibility to scab.....	322, 325	susceptibility to curly-top disease.....	314-316
Peels, apple, quercetin isolation and identification.....	1243-1245	<i>Plasmodiophora brassicae</i> , infection, relation of soil temperature and soil moisture.....	549-562
Pellagra, relation to <i>Oospora verticilloides</i> on corn, discussion.....	918	<i>Platyaster vernalis</i> —	
Peltier, George L., and Frederick, William J.: Further Studies on the Relative Susceptibility to Citrus Canker of Different Species and Hybrids of the Genus Citrus, etc.....	227-239	egg description and development.....	830-832
Relation of Environmental Factors to Citrus Scab Caused by <i>Cladosporium citri</i> Massee.....	241-254	life history and embryonic development.....	829-833
Pests, grain, literature citations.....	1116	polyembryonic development.....	829-849
<i>Pezizella lythri</i> , cause of tan brown rot of strawberries.....	647, 648	<i>Plodia interpunctella</i> , control in warehouses by fumigation.....	649, 659
Pharmacology, gossypol.....	191-198	<i>Pluchea sericea</i> , indicator of land value.....	113, 122, 127
Pharynx, apple maggot larvae.....	8-15	<i>Poa</i> spp., infection with <i>Puccinia graminis poae</i> and inoculation experiments.....	541-542
Phenol, effect on nitrification of cyanamid.....	53-54	Poisoning, gossypol, metabolism of animals.....	192-194
Phlox, <i>Cephalobus</i> occurrence.....	1059-1061	Polish wheat, crosses with <i>vulgare</i> species.....	1019-1031
Phosphorus—		Polynuritis in pigeons, cause, feeding tests.....	464-469
determination in meats.....	340	Polysaccharides in sweet corn.....	427-431
nitride, nitrification, comparison with ammonium sulphate.....	1117-1118	Pomeroy, C. S.: Bud Selection as Related to Quality of Crop in the Washington Navel Orange.....	521-526
Photoperiodism of <i>Tephrosia candida</i> .....	445-460	<i>Poncirus trifoliata</i> , susceptibility to citrus canker.....	227, 230
<i>Physalospora malorum</i> —		Porcupines, injury to young trees, western yellow pine and Douglas fir.....	1143, 1145
comparison with <i>Botryosphaeria ribis</i> .....	596-597	Pore, formation in aecidiospores of <i>Puccinia podophylli</i> .....	925
on currant.....	583-588	Pork, Ascaris-infected hogs, experiments in sensitization.....	579
spores from apple and currant, comparison.....	585-586	Posey, G. B., and Ford, E. R.: Survey of Blister Rust Infection on Pines at Kittery Point, Me., etc.....	1253-1258
<i>Phytophthora cactorum</i> , cause of leather rot of strawberries.....	357, 358-366, 646, 647, 648	Potassium—	
Pickleweed—		di-hydrogen phosphate, effect on wheat growth.....	387-393
association on Southwestern desert soils.....	764-766, 796-797, 800	effect on orange tree growth and composition.....	278, 281, 283
indicator of land value and possibilities.....	111, 118, 121, 122, 126, 127	Potato beetle, insecticides.....	398, 400
Picric acid—		<i>Poteriosotomum</i> spp., parasite of Equidae.....	666, 667
effect on glucose and fructose.....	480-481	Poultry—	
method of sugar determination, modifications.....	479-488	consumption per capita, comparison with other meats.....	461
purifying for sugar tests.....	485-486	flesh, vitamin content.....	461-472
Piemeisel, R. L., and Shantz, H. L.: Indicator Significance of the Natural Vegetation of the Southwestern Desert Region.....	721-802	Powder-post beetle, temperature fatal to, determination.....	1033-1038
Pigeons, feeding with poultry flesh and eggs, experiments.....	462-471	Powick, Wilmer C., and Hoagland, Ralph: The Chemical Composition of Edible Viscera from Meat-Producing Animals.....	339-346
Pima cotton—		Promycella, orange rust on blackberries.....	1047-1050
comparison with Upland varieties in Arizona.....	938-954	Protein, decomposition by <i>Bacillus larvae</i> .....	155-157
fiber, salt content.....	293-295	<i>Puccinia</i> —	
Pine—		<i>graminis</i> —	
lands, central Idaho, growth and reproduction of pine and Douglas fir.....	1139-1147	<i>avena</i> , races, resistance of oat varieties.....	705-720
lumber, growth in Douglas fir stands.....	1237, 1239, 1240	in the United States.....	541-548
reproduction after logging.....	1152-1154, 1157	varieties, comparisons and hosts.....	543-547
western—		<i>podophylli</i> —	
white, germination, relation to surfaces.....	1151-1154	aecidiospores, description, and expulsion method.....	923-926
white, seed, fall sowing and delayed germination.....	1127-1131	mayapple rust, expulsion of aecidiospores.....	923-926
yellow, growth on cut-over and virgin lands in central Idaho.....	1139-1148	spp., rusts of rye and barley, aecial stages.....	1119-1126
yellow, losses in central Idaho, causes.....	1143-1145	Quercetin—	
Pines—		a flavonol pigment in apple peel.....	1243, 1245
blister rust infection at Kittery Point, Me., and effect of <i>Ribes</i> eradication.....	1253-1258	from apple peel, isolation and identification.....	1243-1245
hard, immunity to blister rust.....	74	Rabbitbrush, indicator of land value.....	109, 117, 118, 120-126
infection by blister rust, relation to size and density of stand.....	1255-1257	Rabbits, feeding with gossypol, experiments.....	191-196
		Rainfall—	
		effect on spread of bean disease.....	493-495
		relation to—	
		curly-top.....	302
		strawberry leather rot.....	367-371

	Page		Page
Rands, R. D.: Snails as Predisposing Agents of Sugar Cane "Root Disease" in Louisiana.....	969-970	Rusts—Continued.	
Ransom, B. H.: <i>Ascaris</i> Sensitization.....	577-582	leaf, of rye and barley, aecial stages....	1119-1126
Rations—		orange, blackberry infection.....	1045-1058
steer, winter and summer, composition and amount.....	1217-1218	Rye—	
succulent, comparison with dry rations for steers.....	1231	bacterial blight.....	1039-1040
winter, of steers, effects on pasture gains	1215-1232	brittle straw and other abnormalities....	169-172
Rats—		cell thickness.....	173
feeding—		culms, normal and brittle, analysis.....	170
experiments with cottonseed kernels or gossypol.....	174-186	inbred strains, abnormalities.....	169
with gossypol, experiments.....	176-186	leaf rust, <i>Puccinia dispersa</i> , aecial stages	1119-1126
growth on feed containing gossypol.....	179-185	wild, giant, indicator of land value..	107, 120, 124
Reddy, C. S., Godkin, James, and Johnson, A. G., Bacterial Blight of Rye.....	1039-1040	Sacaton, indicator of land value.....	106, 118, 119, 124, 125
Reed, H. S., and Haas, A. R. C.: Some Relations Between the Growth and Composition of Young Orange Trees and the Concentration of the Nutrient Solution.....	277-284	Sage varieties, indicators of land value....	100-113, 117-126
Reforestation activities, relation to environmental conditions.....	845, 846, 895	Sagebrush, indicator of land value	101, 108, 117-126
Reproduction, forest—		Salt content of cotton fiber.....	293-295
after logging in northern Idaho, factors affecting.....	1149-1157	Salt bush, narrowleaf, association on Southwestern desert soils.....	759-761, 796-799
injury by grazing and other causes.....	1145-1146	Saltgrass, association on southwestern desert soils.....	761-763, 796-799, 800
<i>Rhagoletis pomonella</i> —		Salts, wheat nutrition.....	387-393
larval development.....	1-21	Samphire, indicator of land value.....	111, 118, 121, 122, 125, 126
metamorphosis.....	21-30, 32	Sando, Charles E.: The Isolation and Identification of Quercetin from Apple Peels.	1243-1245
See also Apple maggot; Maggot, apple.		Sap—	
<i>Rhizoctonia</i> brown rot of strawberries, description, and inoculation of fruit.	643-645, 647, 648	cell, density, relation to environmental conditions in the Wasatch Mountains, Utah.....	845-907
<i>Rhizopertha dominica</i> , control in chick-peas.	649, 658	concentration—	
<i>Rhizopus nigricans</i> , presence in cottonseed...	286	relation to osmotic phenomena.....	846-847
<i>Ribes</i> spp.—		seasonal variations.....	888-892
eradication, effect on pine infection by blister rust, survey.....	1253-1258	density—	
spread of blister rust to pines.....	1253-1255, 1257	determination, technique.....	849-851
Rice weevil, resistance to high and low temperatures.....	1043	of plants in Wasatch Mountains.....	860-884
Roberts, John W., and Dunegan, John C.: The Fungus Causing the Common Brown Rot of Fruits in America.....	955-960	orange leaf, concentration and reaction, relative constancy.....	277
Rogues, pea, crosses, results.....	1247-1252	Satsuma oranges—	
Root disease, sugar cane, relation to snails..	969-970	susceptibility to—	
Roots, young orange trees, composition.....	282, 283	citrus canker.....	229-230
Rose, Dean H.: Leather Rot of Strawberries.....	357-376	citrus scab.....	248
Rot—		Sausage tree, African, host of <i>Heterodera radicicola</i> .....	1061
blue mold, citrus fruits, prevention, results of borax treatment.....	961, 968	Sax, Karl, and Gaines, E. F.: A Genetic and Cytological Study of Certain Hybrids of Wheat Species.....	1017-1032
brown—		Scab—	
history of fungus in Europe and America.	955-960	citrus—	
of fruits in America, fungus causing....	955-960	epidemic in Alabama, conditions essential for.....	249, 253
leather—		relation to environmental factors.....	241-254
strawberry.....	357-376	yearly prevalence, conditions determining.....	249-250
strawberry, range and relation to rainfall.....	366-371	pecan—	
onion bulb, cause, and relation to environment.....	683-694	and sources of early spring infections..	321-330
Rots—		description, on leaves, nuts, twigs, catkins, and buds.....	322-324
brown, of strawberries.....	643-648	infection source and control measures..	324-328
<i>Phytophthora</i> , literature.....	374-375	Schwartz, Erich W., and Alsberg, Carl L.: Pharmacology of Gossypol.....	191-198
strawberry—		Relation Between Toxicity of Cottonseed and its Gossypol Content.....	173-189
fruit.....	643-648	Sclerotinia—	
percentage of leather rot to other.....	371-372	<i>cinera</i> , fungus causing brown rot of fruits in America.....	955-960
<i>Rubus</i> spp., infection with orange rusts..	1045-1058	<i>fruticola</i> , same as <i>Sclerotinia cinerea</i> .....	959
Rust—		spp., measurements of asci and ascospores..	957
blister, infection of pines at Kittery Point, Me., and <i>Ribes</i> eradication.....	1253-1258	Scofield, C. S., and Kearney, Thomas H.: The Salt Content of Cotton Fiber.....	293-295
grass, inoculation experiments.....	541-543	Seed—	
mallow, characteristics.....	1045, 1053	coat, permeability gradient to iodine.....	225-226
mayapple, <i>Puccinia podophylli</i> , expulsion of aecidiospores.....	923-926	coats, wheat, means of protection of embryo from hot-water injury.....	86-89
orange—		cotton—	
on blackberry, uninucleated aecidiospores	1045-1058	comparisons.....	950-952
short-cycled, promycelia, characteristic features.....	1049-105	germination.....	285-292
stem, resistance of oat varieties.....	705-720	soaking—	
Rusts—		effects, general.....	489-490
cereal—		literature referring to.....	496-497
literature.....	718-719	western white pine, fall sowing and delayed germination.....	1127-1131
literature relating to.....	547-548	Seedlings—	
physiological races, investigations and discoveries.....	705	Douglas fir, survival under different cutting systems.....	1234-1235
grain, literature.....	1126	orange, growth and composition, effect of concentration of nutrient solution.....	278-279
		Seeds—	
		composites, infestation with <i>Tylenchus dipanci</i> .....	473-478



	Page		Page
Seeds—Continued.		Snodgrass, R. E.: Anatomy and Metamorphosis of the Apple Maggot, <i>Rhagoletis pomonella</i> Walsh	1-36
cotton, prewetting method of germination	287-288	Snyder, T. E., and St. George, R. A.: Determination of Temperatures Fatal to the Powder-post Beetle, <i>Lyctus planicollis</i> Leconte, by Steaming Infested Ash and Oak Lumber in a Kiln	1033-1038
live, in cottonseed sample, demonstration	287	Sodium—	
Seepweed—		cyanid, fumigation of grain for insect pests	1106, 1107, 1108, 1113, 1114, 1115
indicator of land value	111, 118, 121, 122, 125, 126	effect on orange tree growth and composition	278, 281, 283
on southwestern desert soils	773, 778, 796-799	Soil—	
Semi-arid United States, vegetation, types and their significance	99-128	colloids—	
Serological tests, cattle, for infectious abortion	613, 614, 617-620	binding power, factors influencing	499-513
<i>Severinia buxifolia</i> , resistance to citrus canker	228	heat of wetting	927-935
Sewell, M. C.: Relation of the Molecular Proportions in the Nutrient Solution to the Growth of Wheat	387-393	moisture, relation to clubroot	549-562
Sex—		particles, noncolloidal, heat of wetting	929-930
determination in chickens during embryonic development	214-220	temperature, relation to clubroot	549-562
hemp, influence of environment	1067-1080	treatment for control of larkspur leafspot	269
ratio, chickens, experimental study and discussion	200-220	Soils—	
Shadscale, indicator of land value	109, 110, 118, 120, 125	bacteria, effect of cyanamid and related compounds	1159-1166
Shamel, A. D.: Bud Selection as Related to Quality of Crop in the Washington Navel Orange	521-526	colloidal material—	
Shantz, H. L., and—		estimating, heat of wetting as basis	930-934
Aldous, A. E.: Types of Vegetation in the Semi-arid Portion of the United States, etc.	99-128	heat of wetting	928-929
Piemeisel, R. L.: Indicator Significance of the Natural Vegetation of the Southwestern Desert Region	721-802	desert, southwestern, composition and salts	723, 724-725
Sharkey clay soil, colloids from, breaking strength	508-512	salinity, relation to chlorid content of cotton plants	696, 701
Shaw, R. H., and Wright, P. A.: A Study of Ensiling a Mixture of Sudan Grass with a Legume	255-259	Soppeland, Lulu: <i>Flavobacterium suaveolens</i> , a New Species of Aromatic Bacillus Isolated from Dairy Wastes	275-276
Shear, C. L.: Botryosphaeria and Physalospora on Currant and Apple	585-598	Sowing western white pine seed, fall versus spring	1128-1131
Shedd, O. M.: The Determination of Nitrate and Ammonia in Nitrogenous Materials	527-539	Soybeans, silage, composition	258
Sheep, grazing, injury to young coniferous trees	1145-1146	Spacing, cotton, experiments in southern California	1081-1093
Sheets, E. W.: Effect of Winter Rations on Subsequent Pasture Gains of Steers	1215-1232	<i>Sphaeropsis malorum</i> , perfect stage found in currant	584-585
Shelterwood—		Spinach, susceptibility to curly-top	315
cutting method, effect on reproduction	1234, 1235, 1239, 1241, 1242	Spore, discharge, mechanics studies	923, 924, 926
method of cutting Douglas fir, effect on reproduction	1234, 1235, 1237, 1239-1242	Sporocarps of <i>Physalospora malorum</i> , size and structure, relation to host	586-587
Shinnery, indicator of land value	114, 118, 120, 123, 124	Spraying, pecans for scab control	326-327
Shive's nutrient solution, formula	388	Sprays—	
Shoots, young orange trees, composition	382, 383	absorbed organic compounds, insecticidal value	399-400
Shrinkage, steers in transit and in dressing	1230-1231	inorganic nonarsenical compounds, testing as insecticides	396
Shrubs, Wasatch Mountains, freezing-point, depressions, and osmotic pressures	866-871, 878-879	pecan scab	326-327
Silage—		Spring growth, citrus, relation to scab infection	243-253
crops, mixture, typical combinations	255	Spruce, Engelmann, growth in Douglas fir forests	1237-1240
Sudan-grass mixture with legume, palatability and composition	257-258	Squares, cotton, shedding, effect of crazy-top and other diseases	817
Silking, corn, relation to maturity	409-411	Squash lady beetle, insecticides	401
<i>Silvanus cossiae</i> , heating of bran	1105	St. George, R. A., and Snyder, T. E.: Determination of Temperatures Fatal to the Powder-post Beetle, <i>Lyctus planicollis</i> Leconte, by Steaming Infested Ash and Oak Lumber in a Kiln	1033-1038
Silver, James: The European Hare ( <i>Lepus europaeus</i> Pallas) in North America	1133-1137	Stahl, C. F., and Eubanks, Carsner: Studies on Curly-top Disease of the Sugar Beet	297-320
<i>Sitophilus</i> —		Stakman, E. C., and Levine, M. N.: <i>Puccinia graminis poae</i> Erikss. and Henn. in the United States	541-548
granarius. See Weevil, granary; Granary weevil.		Staminate hemp plants, description	1067
oryza—		Star-of-Bethlehem, host of barley leaf rust	1124-1125
heating of grain	1105, 1113, 1115	Steers—	
See also Weevil, rice; Rice weevil.		gains on pasture, effects of winter rations	1215-1232
Skin disease, result of Ascaris poisoning	578, 579, 580, 581	rations, winter and summer, composition and amount	1217-1218
Skinner, J. J.: Greenhouse Experiments with Atmospheric Nitrogen Fertilizers and Related Compounds	971-976	shrinkage in transit and in dressing	1230-1231
Skull, meadow mouse, description and measurement of bones and teeth	992-1014	Steiner, G.: On Some Plant Parasitic Nemas and Related Forms	1059-1066
Skulls, meadow mouse, cleaning and preparing for collection	978	<i>Stellaria media</i> , inoculation with curly-top	312, 313, 315
Slash disposal in cutting Douglas fir, effect on reproduction	1236-1241	Stem rust, resistance of oat varieties	705-720
Smalling, cotton growth disorder	810-811	Stenosis, cotton growth disorder	810-811
Smut, wheat, hot-water treatment modified	79-98	Sterility, wheat hybrids	1017-1032
Snails, relation to root disease of sugar cane in Louisiana	969-970	Stevens, Neil E.—	
Snapdragon, infestation with <i>Heterodera radiculicola</i>	1061	and Dodge, B. O.: The <i>Rhizoctonia</i> Brown Rot and Other Fruit Rots of Strawberries	643-648

	Page		Page
Stevens, Neil E.—Continued.		Tomato, susceptibility to curly-top.....	314
<i>Botryosphaeria</i> and <i>Physalospora</i> on Currant and Apple.....	589-598	Tomosis, cotton growth disorder.....	807-809
<i>Physalospora malorum</i> on currant.....	583-588	Tongues, composition.....	345
Stockton clay adobe soil, colloids from, breaking strength.....	511, 512	Toole, Eben S., and Drummond, Pearl L.: The Germination of Cottonseed.....	285-292
Storage, onion, relation of temperature to rot development.....	691-692, 693	Tracheal system—	
Strawberries—		apple maggot.....	5-7
fruit rots.....	643-648	bee larva.....	1179-1184, 1206
holding tests, rots developing, study of causes.....	373	Trees—	
infection in field with leather rot.....	365-366	hosts of cambium curculio.....	379, 385
inoculation with leather rot.....	364-365	injury by cambium curculio.....	377-386
shipments, by States, 1922.....	367	orange—	
varieties susceptible to leather rot.....	366	analysis of various parts, and ash percentage.....	282, 283
Strawberry leather rot.....	357-376	ash constituents.....	283
<i>Strongylus</i> spp., parasites of Equidae.....	666, 667, 672	dry matter solubility, relation to concentration of solution.....	281-282
Stroud method for nitrate determination.....	528-531	orchard, injury by European hares.....	1136-1137
Sturtevant, A. P.: The Development of American Foulbrood in Relation to the Metabolism of Its Causative Organism.....	129-168	painting wounds to prevent curculio injury.....	385
Sudan grass—		protection against hares.....	1137
mixture—		reserved, in cut-over areas, silvicultural practices.....	1146-1147
with a legume, ensiling.....	255-259	Wasatch Mountains, freezing-point depressions and osmotic pressures.....	861-866, 876-877
with legume silage, composition.....	257-258	young orange, growth and composition, relation to nutrient solution.....	277-284
silage, composition.....	257-258	<i>Trichonema</i> spp., parasites of the Equidae.....	670-671, 672
Sugar—		<i>Triodontophorus</i> spp., parasites of the Equidae.....	666, 667
content, sweet corn, changes and tests.....	427, 434-435	<i>Triticum</i> —	
determination, literature.....	487-488	<i>dicoccum dicoccoides</i> , genetic relation to a synthetic form.....	515-520
in bee larvae, quantitative determination.....	141-150, 157-159	<i>vulgare</i> , cross with a variety of <i>Triticum durum</i> .....	515, 519
Sugars—		Trunk, young orange trees, composition.....	282, 283
color values, comparison with glucose.....	484-485	Tubercles, plantar, on foot of meadow mouse, variation.....	983, 1014
determination, picric acid methods.....	479-488	Tuckwiller, David: Steer-fattening experiments.....	1215
Tall, meadow mouse, measurement.....	987	Turkeys, flesh, vitamin B content.....	463, 468, 472
Tan brown rot of strawberries, description.....	645-646, 648	Turnips, injury by clubroot.....	551
Tapke, V. F.: Effects of the Modified Hot-water Treatment on Germination, Growth, and Yield of Wheat.....	79-98	Tussock grass, indicator of land value.....	106, 119, 125, 127
<i>Taraxacum officinale</i> . See Dandelion.		Twig, pecan, scab description and source of infection.....	324
Taylor, J. W., and Leighty, C. E.: Hairy-neck Wheat Segregates from Wheat-Rye Hybrids.....	567-576	Typhoid, fowl, dissemination and control.....	75-78
Teeth, meadow mouse, description, measurements, and variations.....	1010-1013, 1015	Ulsch-Street method of nitrate determination, comparisons.....	527, 533-538
Teliospores, <i>Puccinia dispersa</i> , germination.....	1120-1122	<i>Uncinaria stenocephala</i> , occurrence in foxes.....	331
Temperature—		Upland cotton—	
effect on spread of bean disease.....	493-495	chlorid content of leaf tissue, comparison with Egyptian.....	695-704
fluctuations in duff and bare soil, comparisons.....	1154-1156	susceptibility to crazy-top.....	824, 826
relation to—		varieties, comparison with Pima cotton in Arizona.....	938-954
citrus scab infection and development.....	243-253	Weed, Star-of-Bethlehem, danger near barley fields.....	1125, 1126
curly-top.....	313	Weevils—	
leather rot of strawberries.....	369, 371, 372-373	bean—	
onion bulb rot, occurrence and distribution.....	689-691	effect of fumigation.....	348-349
Temperatures—		egg, effect of fumigation.....	350-351
fatal to powder-post beetle, in ash and oak lumber.....	1033-1038	fumigation.....	347-356
greenhouse, in wheat growing experiments.....	389	resistance to high and low temperatures.....	1043-1044
high and low, resistance of rice and granary weevils.....	1043-1044	within bean seeds, effect of fumigation.....	351-355
Teosinte, chromosome number.....	673-676, 678	Weight, meadow mice, variations.....	988-989
Teosinte-and-corn hybrids, chromosome number.....	674-676	West Virginia, steers, fattening.....	1217-1218
<i>Tephrosia candida</i> —		Western blight. See Curly-top.	
growth and blossoming, relation to length of day.....	445-460	Wetting, heat of soil colloids.....	927-935
photoperiodism.....	445-460	Wheat—	
Tests, corn, canning quality.....	431-433	cross, inheritance of pubescent nodes.....	841-844
Texas, cottonseed, germination studies.....	285-292	crosses, literature relating to.....	572, 574, 575-576
<i>Thersilochus conotracheli</i> , parasite of cambium curculio.....	385	emmer species, crosses with <i>vulgare</i> species.....	1018-1031
Thompson, H. E.: Brittle Straw and Other Abnormalities in Rye.....	169-172	fertilizer experiments.....	972-975
Thumb-nail test of corn.....	435	genetics, literature.....	1031, 1032
Timber, woodland, indicator of land value.....	116, 120, 123-126	germination, effect of modified hot-water treatment.....	80-89
Tims, E. C., and Walker, J. C.: A Fusarium Bulb Rot of Onion and the Relation of Environment to Its Development.....	683-694	groups, classification and characteristics.....	1017
<i>Tipsacum dactyloides</i> , chromosome number.....	677-678	growth—	
Tobacco—		and yield of modified hot-water treatment.....	89-95
grass, indicator of land value.....	105, 118, 124, 125	relation to salts in nutrient solution.....	387-393
nitrate determination methods.....	531-533	"hairly neck," segregates from wheat-rye hybrids.....	567-576
Tom Thumb maize, crossing with Jala maize.....	1095-1102	heating caused by insects.....	1103-1104, 1105, 1107-1109, 1113-1115



	Page		Page
Wheat—Continued.		Varnette, D. L.: Comparison of Pima Cotton with Upland Varieties in Arizona.....	937-954
hot-water treatment, modified, effect on germination, growth, and yield.....	79-98	Vegetation—	
hybrids—		desert region, indicator significance.....	721-802
sterility, causes.....	1017-1032	semiarid, in United States, types and significance.....	99-128
study, genetic and cytological.....	1017-1032	type, western United States, regions and key map.....	121-127
salt nutrition.....	387-393	types—	
seed coats, permeability to iodine.....	225-226	agricultural possibilities.....	117-119
species hybrids, characters association.....	1025-1027	relation to character and productivity of the land.....	795-800
treatment literature.....	97	zones in mountainous regions of the West.....	845
<i>vulgare</i> species, crosses with emmer species.....	1018-1031	Velvetnode wheat, heredity studies.....	841-844
yield, effects of modified hot-water treatment.....	90-95	Virus, curly-top, transmission methods and factors affecting.....	306-316
Wheatgrass, indicator of land value.....	100, 102, 103, 117, 120, 124	Viscera, edible, of meat-producing animals, chemical composition.....	339-346
Wheat-rye hybrids—		Vitamin—	
hairy neck segregates.....	567-576	antineuritic, in poultry flesh and eggs.....	461-472
self sterility.....	567-568, 573-575	B, content of dried poultry flesh and eggs.....	462-472
Wilcox, Marguerite S.: <i>Botryosphaeria</i> and <i>Phylospora</i> on Currant and Apple.....	585-598	Vliet, E. B.: Greenhouse Experiments with Atmospheric Nitrogen Fertilizers and Related Compounds.....	971-976
Willaman, J. J., and Davison, F. R.: Some Modifications of the Pieric Acid Method for Sugars.....	479-488	<i>Vulgare</i> wheat group, characters.....	1017
Willow, indicator of land value.....	114, 118, 119, 123, 124, 125, 126	Wahlenberg, W. G.: Fall Sowing and Delayed Germination of Western White Pine Seed.....	1127-1131
Wilt, bean, causes and spread.....	490-493	Walker, J. C., and Tims, E. C.: A <i>Fusarium</i> Bulb Rot of Onion and the Relation of Environment to its Development.....	683-694
Windfall, losses caused to western yellow pine and Douglas fir.....	1143-1145	War gases, utilization as insecticides.....	399, 400
Winds, storm, spread of blister rust to pines.....	1253-1255, 1257	Warehouses, grain, fumigation experiments with chick-peas.....	650-659
Wineland, Grace O.: An Ascigerous Stage and Synonymy for <i>Fusarium moniliforme</i> .....	909-922	Wasatch—	
Winter rations of steers, effects on pasture gains.....	1215-1232	Mountain region, vegetation, temperature, precipitation, etc.....	851-860
Winter-fat, indicator of land value.....	110, 118, 120, 125, 126	Mountains, Utah, environmental conditions, relation to density of cell sap.....	845-907
Wisconsin, clubroot studies.....	549	Washington—	
Worms, control in foxes.....	334-337	Experiment Station wheat hybrids, growing.....	1019
Wounds, tree, infestation with cambium curculio.....	379-385	Hybrid 143, origin, and crosses with emmer.....	1019-1031
Wright, P. A., and Shaw, R. H.: A Study of Ensiling a Mixture of Sudan Grass With a Legume.....	255-259	Wastes, dairy <i>Flavobacterium suaveolens</i> , a new species of aromatic bacillus.....	275-276
Urea—		Water—	
effect on numbers of bacteria in soil.....	1160, 1161, 1165, 1166	soak. See Rot, leather, strawberry.	
investigations and discussions.....	37-39	supply, relation to sap density of leaves and roots.....	885-888
nitrification rates and decomposition in soil.....	43, 48-54	Weather, effect on citrus scab distribution and prevalence.....	250-252
nitrogen, determination method.....	42	Yolk, egg, weight and water content, relation to sex ratio.....	211-213
Urephos, fertilizer tests.....	973-975	Yucca—	
<i>Uromyces hordei</i> , rust of wild barley and its aecial stage.....	1124	association with cactus on Southwestern desert soils.....	731-733, 797
Utah, Wasatch Mountains, environmental conditions of trees, relation to density of cell sap.....	845-907	indicator of land value.....	103, 114
Vaccination—		Zebra—	
cattle, to control infectious abortion.....	609-620	nematode parasite, <i>Cylindropharynx ornata</i> , and others.....	661-672
fowls, for control of typhoid.....	78	parasites.....	666-672
Vaccine, abortion, immunization of cattle.....	609-620	<i>Zinnia elegans</i> roots infestation by <i>Paratylenchus nanus</i> .....	1064-1065
Van Volkenberg, H. L., and Hanson, Karl B.: Anthelmintic Efficiency of Carbon Tetrachlorid in the Treatment of Foxes.....	331-337		